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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
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☑ *Additional inventors are being named on the* 1 *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Methods and Compositions for the Diagnosis of Neuroendocrine Lung Cancer

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS (*check all that apply*)

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☐ Application Data Sheet. See 37 CFR 1.76			

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☐ Applicant claims small entity status. See 37 CFR 1.27.		FILING FEE AMOUNT ($)
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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.

☑ Yes, the name of the U.S. Government agency and the Government contract number are: DHHS, NIH, CCR

Respectfully submitted,

SIGNATURE

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Date 11/04/2002

REGISTRATION NO. *(if appropriate)* 32,680

Docket Number: 03514.108

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

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Docket Number	03514.108

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Number 1 of 1

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Title of the Invention:

Methods and Compositions for the Diagnosis of Neuroendocrine Lung Cancer

Field of the Invention:

This invention relates to methods and compositions for the diagnosis of neuroendocrine lung cancers. In particular, the invention concerns the use of cDNA microarrays to facilitate the differential diagnosis of neuroendocrine tumor types

Statement of Governmental Interest

This invention was funded by NCI Intramural Research Program CCR at the National Institutes of Health. The United States Government has certain rights to this invention.

Background of the Invention:

The mammalian neuroendocrine system is a dispersed organ system that consists of cells found in multiple different organs. The cells of the neuroendocrine system function in certain ways like nerve cells and in other ways like cells of the endocrine (hormone-producing) glands. The neuroendocrine cells of the lung are of particular significance; they help control airflow and blood flow in the lungs and may help control growth of other types of lung cells.

In some instances, neuroendocrine cells escape from normal cellular control and become malignant, resulting in neuroendocrine tumors. Four clinically distinct types of neuroendocrine tumors have been described: small cell lung cancer (SCLC), large cell neuroendocrine carcinoma (LCNET), typical carcinoid (TC) tumors and atypical carcinoid (AT) tumors. SCLC is the most serious type of neuroendocrine lung tumor (LCNEC), and is among the most rapidly growing and spreading of all cancers. Large cell neuroendocrine carcinoma, typical carcinoid

and atypical carcinoid tumors are rare forms of cancers. Whereas SCLC accounts for 15-25% of total pulmonary malignancies, large cell neuroendocrine carcinoma, typical carcinoid and atypical carcinoid tumors collectively account for only 3-5% of total pulmonary malignancies. Accurate diagnosis of neuroendocrine carcinoma is important since the different tumor types are associated with markedly different survival rates. Small Cell Lung Cancers are associated with 5 and 10 year survival rates of only 9% and 5%, respectively. Large Cell Neuroendocrine Carcinoma presently exhibit 27% and 9%, 5 and 10 year survival rates. Atypical Carcinoid Tumors are associated with 5 and 10 year survival rates of 56% and 35%, respectively. In contrast, Typical Carcinoid Tumors are found to have 5 and 10 year survival rates of nearly 90%

Neuroendocrine tumors are reviewed by Gould, V.E. *et al.* (2000) "EPITHELIAL TUMORS OF THE LUNG" *Chest Surg Clin N Am 10*:709-28, by DeLellis, R.A. (1997) "PROLIFERATION MARKERS IN NEUROENDOCRINE TUMORS: USEFUL OR USELESS? A CRITICAL REAPPRAISAL" *Verh Dtsch Ges Pathol. 81*:53-61, by Travis, W.D. *et al.* (1991) "NEUROENDOCRINE TUMORS OF THE LUNG WITH PROPOSED CRITERIA FOR LARGE-CELL NEUROENDOCRINE CARCINOMA. AN ULTRASTRUCTURAL, IMMUNOHISTOCHEMICAL, AND FLOW CYTOMETRIC STUDY OF 35 CASES" *Am J Surg Pathol 15*:529-53, by Cerilli, L.A. *et al.* (2001) "NEUROENDOCRINE NEOPLASMS OF THE LUNG" Am J Clin Pathol 116:S65-96; by Arrigoni, M.G. *et al.* (1972) "ATYPICAL CARCINOID TUMORS OF THE LUNG," J Thorac Cardiovasc Surg 64:413-421; by Warren, W.H. *et al.* (1988) "WELL DIFFERENTIATED AND SMALL CELL NEUROENDOCRINE CARCINOMAS OF THE LUNG: TWO RELATED BUT DISTINCT CLINICOPATHOLOGIC ENTITIES," Virchows Arch B cell Pathol 55:299-310; by Kramer, R. (1930) "ADENOMA OF BRONCHUS," Ann Otol Rhinol Laryngol 39:689, and by Mark, E.J. *et al.* (1985) "PERIPHERAL SMALL CELL CARCINOMA OF THE LUNG RESEMBLING CARCINOID TUMOR," Arch Pathol Lab Med 109:263-269.

Unfortunately, all neuroendocrine tumors have similar morphologic appearances and exhibit similar immunohistochemical staining. Thus, a significant

difficulty presently exists in accurately distinguishing between the different types of neuroendocrine tumors. Such diagnosis is still "decisively" based on light-microscopic evaluations of tissue samples for the number of cells involved in mitosis. Other than clinical stage at presentation, mitotic count is currently the sole independent histologic predictor of prognosis (Junker, K. *et al.* (2000) "PATHOLOGY OF SMALL-CELL LUNG CANCER," *J Cancer Res Clin Oncol. 126*:361-8; Franklin, WA. (2000) "PATHOLOGY OF LUNG CANCER" *J Thorac Imaging. 15*:3-12; Chyczewski, L. *et al.* (2001) "MORPHOLOGICAL ASPECTS OF CARCINOGENESIS IN THE LUNG" *Lung Cancer. 34*:S17-25; Travis, W.D. *et al.* (1991) "NEUROENDOCRINE TUMORS OF THE LUNG WITH PROPOSED CRITERIA FOR LARGE-CELL NEUROENDOCRINE CARCINOMA. AN ULTRASTRUCTURAL, IMMUNOHISTOCHEMICAL, AND FLOW CYTOMETRIC STUDY OF 35 CASES" *Am J Surg Pathol 15*:529-53; Brambilla, E. *et al.* (2001) "THE NEW WORLD HEALTH ORGANIZATION CLASSIFICATION OF LUNG TUMOURS" *Eur Respir J. 18:*1059-68).

Such microscopic evaluations of tissue samples is complex and difficult. Moreover, no "gold-standard" exists for defining neuroendocrine differentiation (Carnaghi, C. *et al.* (2001) "CLINICAL SIGNIFICANCE OF NEUROENDOCRINE PHENOTYPE IN NON-SMALL-CELL LUNG CANCER" *Ann Oncol. 12*:S119-23). The absence of an effective diagnostic standard complicates the management and treatment of neuroendocrine tumors (Obërg, K. (2001) "CHEMOTHERAPY AND BIOTHERAPY IN THE TREATMENT OF NEUROENDOCRINE TUMOURS," *Ann Oncol 12*:S111-4).

Researchers have attempted to apply the principles of molecular biology in order to identify molecular markers that would facilitate the diagnosis of neuroendocrine tumor types (see, for example, Japanese Patent Document JP 58,198,758A2; and United States Patents Nos. 5,766,888; 5,856,097; 5,866,323; 5,965,362; 5,976,790; 5,985,240; 5,998,154; 6,132,724; 6,166,176; 6,180,082; 6,225,049; 6,238,877; 6,251,586; 6,335,167; and 6,358,491). Certain proteins, such as chromogranin A (CgA) and neuron-specific enolase (NSE) have been identified as having specific potential use in the clinical diagnosis of

neuroendocrine tumors (Seregni, E. *et al.* (2000) "LABORATORY TESTS FOR NEUROENDOCRINE TUMOURS" *Q J Nucl Med. 44*:22-41). Non-SCLC neuroendocrine tumors have been reported to overexpress CgA whereas SCLC tumors exhibit elevated NSE levels. *Id.* Lui, W.-O. *et al.* (2001) "HIGH LEVEL AMPLIFICATION OF 1P32-33 AND 2P22-24 IN SMALL CELL LUNG CARCINOMAS" *Intl. J Oncol. 19*:451-457 used comparative genomic hybridization analysis to identify chromosomal abnormalities in SCLC tumor cells. Through such analysis, several genetic regions were found to be amplified (i.e., 1p32, 2p23, 1p32, and 2p32). A loss of heterozygosity (LOH) is observed on 3p, 13q and 17p in nearly all SCLC tumors (Yokota *et al.* (1987) "LOSS OF HETEROZYGOSITY ON CHROMOSOMES 3, 13 AND 17 IN SMALL CELL CARCINOMA AND ON CHROMOSOME 3 IN ADENOCARCINOMA OF THE LUNG" *Proc. Natl. Acad. Sci. (U.S.A.) 84*:9252-9256. Similarly, deletions in 11q have been correlated with the presence of AT and TC tumors (Walch, A.K. *et al.* (1998) "TYPICAL AND ATYPICAL CARCINOID TUMORS OF THE LUNG ARE CHARACTERIZED BY 11Q DELETIONS AS DETECTED BY COMPARATIVE GENOMIC HYBRIDIZATION" *Am J Pathol. 153*:1089-98).

While such efforts have succeeded in identifying quantitative differences in mutations affecting various genes (for example finding that p53 is inactivated in >90% of SCLC tumors, but in only >50% of non-SCLC tumors, or that p16 abnormalities arise in <1% of SCLC tumors but in ~66% of non-SCLC tumors), clear correlations that would support a definitive differential diagnosis of tumor type has not been fully achieved (see, Ignacio, I. *et al.* (2001) "MOLECULAR GENETICS OF SMALL CELL LUNG CARCINOMA" *Semin Oncol. 28*:3-13; Carnaghi, C. *et al.* (2001) "CLINICAL SIGNIFICANCE OF NEUROENDOCRINE PHENOTYPE IN NON-SMALL-CELL LUNG CANCER" *Ann Oncol. 12*:S119-23). In this regard, one recent study found no statistically significant correlation between any individual marker and response to chemotherapy for non-SCLC tumors (Gajra, A. *et al.* (2002) "THE PREDICTIVE VALUE OF NEUROENDOCRINE MARKERS AND P53 FOR RESPONSE TO CHEMOTHERAPY AND SURVIVAL IN PATIENTS WITH ADVANCED NON-SMALL CELL LUNG CANCER" Lung Cancer. 36:159-65). Thus, a need remains for a usable

molecular marker approach that could distinguish between the different types of neuroendocrine tumors.

cDNA microarrays have been employed to analyze gene expression patterns in human cancers (DeRisi, J. *et al.* (1996) "USE OF A CDNA MICROARRAY TO ANALYSE GENE EXPRESSION PATTERNS IN HUMAN CANCER" *Nature Genetics 14*:457-60). Such efforts have combined DNA amplification technologies (such as T7-based RNA amplification) with cDNA microarrays in order to improve the discriminating power of the analysis (Luo, L. *et al.* (1999) "GENE EXPRESSION PROFILES OF LASER-CAPTURED ADJACENT NEURONAL SUBTYPES" *Nature Medicine 5*:117-22; Bonner, R.F. *et al.* (1997) "LASER CAPTURE MICRODISSECTION: MOLECULAR ANALYSIS OF TISSUE" *Science 278*:1481,1483; Schena, M. *et al.* (1995) "QUANTITATIVE MONITORING OF GENE EXPRESSION PATTERNS WITH A COMPLEMENTARY DNA MICROARRAY" *Science 270*:467-70).

Despite all such progress, no fully successful method for distinguishing between the neuroendocrine tumor types, and of thus accurately diagnosing neuroendocrine cancers has yet been achieved. The present invention is, in part, directed to such needs.

Summary of the Invention:

This invention relates to methods and compositions for the diagnosis of neuroendocrine lung cancers. The present invention permits one to accurately classify pulmonary neuroendocrine tumors based on their genome-wide expression profile without further manipulation. A hierarchical clustering of all genes classifies these tumors according to World Health Organization (WHO) histological type. The selection of genes with significant variance resulted in the identification of 198 transcripts, many of which have potentially important biological and clinical implications. The present invention thus defines and provides groups of genes that identify each tumor type, and permits one to derive a molecular signature that distinguishes each subtype of neuroendocrine tumor.

In detail, the invention provides a method for determining whether a candidate cell is a neuroendocrine tumor cell, wherein the method comprises the steps of:

(A) determining the profile of expression of a plurality of genes of the candidate cell; and

(B) comparing such determined profile of expression with the profile of expression of the genes of a small cell lung cancer cell, a large cell neuroendocrine carcinoma cell, a typical carcinoid tumor cell or an atypical carcinoid tumor cell;

to thereby determine whether the candidate cell is a neuroendocrine tumor cell.

The invention particularly concerns the embodiment of such method wherein the method additionally permits a determination of neuroendocrine tumor cell type. The invention further concerns the embodiments of such methods wherein the method determines whether the candidate cell is a small cell lung cancer (SCLC) neuroendocrine tumor cell, a large cell neuroendocrine carcinoma (LCNEC) neuroendocrine tumor cell, a typical carcinoid (TC) neuroendocrine tumor cell, or an atypical carcinoid (AT) neuroendocrine tumor cell.

The invention further concerns the embodiments of such methods wherein step (A) of the methods comprise incubating RNA of the candidate cell, or DNA or RNA amplified from such RNA, in the presence of a plurality of genes, or fragments or RNA transcripts thereof, under conditions sufficient to cause RNA to hybridize to complementary DNA or RNA molecules; and detecting hybridization that occurs.

The invention additionally concerns the embodiments of such methods wherein the plurality of genes, or polynucleotide fragments or RNA transcripts thereof, are distinguishably arrayed in a microarray. The invention particularly concerns the embodiments of such methods wherein the microarray comprises

arrayed genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in neuroendocrine tumor cells relative to normal cells.

The invention particularly concerns the embodiments of such methods wherein the microarray comprises arrayed genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in small cell lung cancer (SCLC) neuroendocrine tumor cells relative to large cell neuroendocrine carcinoma (LCNEC) neuroendocrine tumor cells.

The invention particularly concerns the embodiments of such methods wherein the microarray comprises arrayed genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in small cell lung cancer (SCLC) neuroendocrine tumor cells relative to typical carcinoid (TC) neuroendocrine tumor cells.

The invention particularly concerns the embodiments of such methods wherein the microarray comprises arrayed genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in small cell lung cancer (SCLC) neuroendocrine tumor cells relative to atypical carcinoid (AT) neuroendocrine tumor cells.

The invention particularly concerns the embodiments of such methods wherein the microarray comprises arrayed genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in large cell neuroendocrine carcinoma (LCNEC) neuroendocrine tumor cells relative to typical carcinoid (TC) neuroendocrine tumor cells.

The invention particularly concerns the embodiments of such methods wherein the microarray comprises arrayed genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in large cell neuroendocrine carcinoma (LCNEC) neuroendocrine tumor cells relative to atypical carcinoid (AT) neuroendocrine tumor cells.

The invention particularly concerns the embodiments of such methods wherein the microarray comprises arrayed genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in typical carcinoid (TC) neuroendocrine tumor cells relative to atypical carcinoid (AT) neuroendocrine tumor cells

The invention additionally concerns a microarray of genes, or polynucleotide fragments or RNA transcripts thereof for distinguishing a neuroendocrine tumor cell, the microarray comprising a solid support having greater than 10 genes, or polynucleotide fragments or RNA transcripts thereof, distinguishably arrayed in spaced apart regions, wherein the microarray comprises a sufficient number of genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in a small cell lung cancer (SCLC) cell, a large cell neuroendocrine carcinoma (LCNEC) neuroendocrine tumor cell, a typical carcinoid (TC) neuroendocrine tumor cell, or an atypical carcinoid (AT) neuroendocrine tumor cell, relative to a normal cell or a cell belonging to a different neuroendocrine tumor cell type, to permit the microarray to distinguish a pulmonary neuroendocrine tumor cell.

The invention particularly concerns the embodiment of such microarray wherein the microarray comprises a sufficient number of genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in a neuroendocrine tumor cell relative to a normal cell to permit the microarray to distinguish between a neuroendocrine tumor cell and a normal cell.

The invention particularly concerns the embodiments of such microarrays wherein the microarray comprises a sufficient number of genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in a small cell lung cancer (SCLC) neuroendocrine tumor cell relative to a large cell neuroendocrine carcinoma (LCNEC) neuroendocrine tumor cell to permit the microarray to distinguish between a small cell lung cancer (SCLC) neuroendocrine

tumor cell and a large cell neuroendocrine carcinoma (LCNEC) neuroendocrine tumor cell.

The invention particularly concerns the embodiments of such microarrays wherein the microarray comprises a sufficient number of genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in a small cell lung cancer (SCLC) neuroendocrine tumor cell relative to a typical carcinoid (TC) neuroendocrine tumor cell to permit the microarray to distinguish between a small cell lung cancer (SCLC) neuroendocrine tumor cell and a typical carcinoid (TC) neuroendocrine tumor cell.

The invention particularly concerns the embodiments of such microarrays wherein the microarray comprises a sufficient number of genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in a small cell lung cancer (SCLC) neuroendocrine tumor cell relative to an atypical carcinoid (AT) neuroendocrine tumor cell to permit the microarray to distinguish between a small cell lung cancer (SCLC) neuroendocrine tumor cell and an atypical carcinoid (AT) neuroendocrine tumor cell.

The invention particularly concerns the embodiments of such microarrays wherein the microarray comprises a sufficient number of genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in a large cell neuroendocrine carcinoma (LCNEC) neuroendocrine tumor cell relative to a typical carcinoid (TC) neuroendocrine tumor cell to permit the microarray to distinguish between a large cell neuroendocrine carcinoma (LCNEC) neuroendocrine tumor cell and a typical carcinoid (TC) neuroendocrine tumor cell.

The invention particularly concerns the embodiments of such microarrays wherein the microarray comprises a sufficient number of genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in a large cell neuroendocrine carcinoma (LCNEC) neuroendocrine tumor cell relative to an atypical carcinoid (AT) neuroendocrine tumor cell to permit the microarray to distinguish between a large cell neuroendocrine carcinoma (LCNEC)

neuroendocrine tumor cell and an atypical carcinoid (AT) neuroendocrine tumor cell.

The invention particularly concerns the embodiments of such microarrays wherein the microarray comprises a sufficient number of genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in a typical carcinoid (TC) neuroendocrine tumor cell relative to an atypical carcinoid (AT) neuroendocrine tumor cell to permit the microarray to distinguish between a typical carcinoid (TC) neuroendocrine tumor cell and an atypical carcinoid (AT) neuroendocrine tumor cell.

Brief Description of the Figures:

Figures 1A-1D illustrate carcinoid tumor tissue sections before and after laser capture microdissection; H&E (**Figure 1A**); Before LCM (**Figure 1B**); After LCM (**Figure 1C**); Dissected Tissue on the cap (**Figure 1D**).

Figure 2 shows the hierarchical clustering of genes with statistically significant variance ($p<0.004$) among all tumor samples.

Figure 3 shows the hierarchical clustering of 198 genes, created by enforcing the classification of 17 tumors.

Figures 4A and **4B** show the expression of genes of large cell neuroendocrine tumor cells and typical carcinoid tumor cells.

Description of the Preferred Embodiments:

The invention concerns methods and compositions for the diagnosis of neuroendocrine lung cancers. Lung cancer is a leading cause of cancer-related deaths (Franceschi, S. *et al.* (1999) "THE EPIDEMIOLOGY OF LUNG CANCER," Ann. Oncol. 10 Suppl 5:S3-6). The observed continuous relative increase in the incidence of SCLC (Junker, K. *et al.* (2000) "Pathology of Small-Cell Lung Cancer, J. Cancer Res. Clin. Oncol. 126:361-368) reflects cigarette smoking, lack of effective methods for early diagnosis and paucity of information on phenotypic

changes which predict the development of aggressive types of lung cancer. Neuroendocrine tumors are a distinct subset of lung cancers that share morphologic, ultrastructural, immunohistochemical, and molecular characteristics. As indicated above, the term neuroendocrine tumors encompasses small cell lung cancer (SCLC) tumors, large cell neuroendocrine carcinomas, typical carcinoid (TC) tumors and atypical carcinoid (AT) tumors. All neuroendocrine tumors have similar morphologic appearance with organoid, trabecular or rosettelike pattern; immunohistochemical staining for chromogranin (Cga), synaptophysin, neuron-specific enolase (NSE), neural cell adhesion molecule (NCAM), and the presence of neuroendocrine granules, which can be detected by electron microscopy (Fisher, E.R. *et al.* (1978) "COMPARATIVE HISTOPATHOLOGIC, HISTOCHEMICAL, ELECTRON MICROSCOPIC AND TISSUE CULTURE STUDIES OF BRONCHIAL CARCINOIDS AND OAT CELL CARCINOMAS OF THE LUNG," Am J Clin Pathol 69: 165-172).

The dramatic differences in survival exhibited by the different neuroendocrine malignancies reflect fundamental differences in biological behavior and therapeutic approaches in these tumors (Travis, W.D., *et al.* (1998) "SURVIVAL ANALYSIS OF 200 PULMONARY NEUROENDOCRINE TUMORS: WITH CLARIFICATION OF CRITERIA FOR ATYPICAL CARCINOID AND ITS SEPARATION FROM TYPICAL CARCINOID," Am J Surg Pathol 22:934-944). Current treatment for patients with TC involves surgical resection because the tumors are slow growing and frequently detected as solitary pulmonary lesions. In less than one third of patients with LCNEC, surgical resection is possible without neoadjuvant treatment. Unfortunately, at the time of diagnosis, most SCLC tumors are disseminated, treatment is not effective and the prognosis is poor. Thus, accurate diagnosis of each type of pulmonary neuroendocrine tumors is essential for successful clinical outcome.

The combined use of light microscopy, immunohistochemistry and electron microscopy has increased the oncologist's ability to differentiate different subtypes of neuroendocrine tumors and has provided clues regarding their pathogenesis. However, little information is available on genetic changes associated with each type of neuroendocrine tumors.

Over the past decade, there have been significant changes in the classification of pulmonary neuroendocrine tumors in order to improve prediction of their biological behavior. The accurate diagnosis of each pulmonary tumor subtype is critical for decisions of therapy. A diagnosis based on light microscopic examination, specifically in differentiation of SCLC from other pulmonary NETs is often challenging. Unfortunately, there are no molecular markers to aid in differentiation of each tumor subtype.

In accordance with the methods of the present invention, the analysis of genome-wide gene expression in neuroendocrine tumors from cDNA microarray data (often referred to as "unsupervised learning") accurately distinguishes each tumor type. The pattern of gene expression has been found to correlate with each subtype assigned by light microscopy according to WHO/LASLSC classification (Histopathological classification of these tumors is based on the 1999 WHO Classification (Travis, W.D. *et al.* (1999) "HISTOLOGIC TYPING OF LUNG AND PLEURAL TUMORS" (Ed 3). Berlin, Germany, Springer).

Microarray technology is widely used to identify changes in gene expression accompanying altered cell physiology during development, cell cycle progression, drug treatment or disease progression. Related phenotypes are usually accompanied by related patterns of cellular transcripts that can be used to characterize these changes. The present invention exploits the recent development of DNA microarray technology (see, for example, DeRisi, J. *et al.* (1996) "USE OF A cDNA MICROARRAY TO ANALYSE GENE EXPRESSION PATTERNS IN HUMAN CANCER" *Nature Genetics 14*:457-60; Luo, L. *et al.* (1999) "GENE EXPRESSION PROFILES OF LASER-CAPTURED ADJACENT NEURONAL SUBTYPES" *Nature Medicine 5*:117-22; Bonner, R.F. *et al.* (1997) "LASER CAPTURE MICRODISSECTION: MOLECULAR ANALYSIS OF TISSUE" *Science 278*:1481,1483; Schena, M. *et al.* (1995) "QUANTITATIVE MONITORING OF GENE EXPRESSION PATTERNS WITH A COMPLEMENTARY DNA MICROARRAY" *Science 270*:467-70) to analyze genome-wide changes that may distinguish these tumors and discover molecular markers. The identification of such markers and their subsequent use

ion the diagnosis and monitoring of neuroendocrine cancers permits a more effective selection of treatment modalities for individual patients.

The analysis of changes in gene expression in clinical specimens is complicated by the mixture of tumor and normal cells, as well as stromal, vascular, and other cells obtained in biopsy. In addition, the heterogeneity of cell type hinders the study of gene expression profiles in cancer cells. Although the principles of the present invention may be used with tissue biopsies and other tissue samples, most preferably, the analysis will be conducted with single cells. Such single cells can be isolated using any of a variety of methods, however, the use of laser capture microdissection (LCM) is preferred. Laser capture microdissection is a procedure that permits the harvesting of a specific cell population directly from frozen sections. The procedure involves fixing the desired cells to a thermoplastic film following infrared laser pulse to avoid "contamination" by other cell populations (Emmert-Buck, M.R. *et al.* (1996) "Laser Capture Microdissection," Science 274:998-1001; Goldsworthy, S.M. *et al.* (1999) "Effects Of Fixation On RNA Extraction And Amplification From Laser Capture Microdissected Tissue," Molecular Carcinogenesis, 1999, 86-91; Luo, L. *et al.* (1999) "Gene Expression Profiles Of Laser-Captured Adjacent Neuronal Subtypes" *Nature Medicine* 5:117-22).

Most preferably, the PixCell™ LCM system (Arcturus, Moutain View, CA) is used for laser capture microdissection (Bonner, R.F., *et al.* (1997) "Laser Capture Microdissection: Molecular Analysis Of Tissue," Science 278: 1481,1483). The examples described below illustrate the desirability of isolating tumor cells from vascular and inflammatory components frequently found in surgical specimens of lung cancer and other vascular tumors.

The present invention thus permits one to distinguish between different neuroendocrine tumor subtypes based on their expression profiles. Preferably, such analysis will involve a comparison of the expression of multiple genes, and is accomplished by assessing the extent or presence of hybridization occurring

between RNA transcripts (or cDNA copies thereof) of a candidate cell and genes, or polynucleotide fragments or RNA transcripts thereof of a reference cell that are differentially expressed in some or all neuroendocrine tumor cells. As used herein, a gene is said to be "differentially expressed" in a tumor cell if detection of its expression facilitates the determination that a candidate cell is or is not a tumor cell.

Clones containing suitable genes, and from which suitable polynucleotide fragments or RNA transcripts can be made, are obtainable from Incyte Genomics (www.incyte.com). The present invention provides a preferred set of 198 genes that are particularly suited for use in such analysis. Clones of these genes are commercially available from Incyte Genomics using the Incyte Clone ID No. information provided in **Table 2**. Preferably the analysis will be conducted using 10%, 20%, 50%, 70%, 80%, 90% or all of these 198 genes, alone or in combination with other genes, or polynucleotide fragments or RNA transcripts thereof. These 198 genes have been found to define three different cluster groups. The analysis may involve a comparison of the expression of genes belonging to the same cluster group, or to two or more different cluster groups.

cDNA microarrays are preferably performed on a solid surface, such as a chip or slide. Preferably, such surfaces will contain multiple human genes, or polynucleotide fragments or RNA transcripts thereof, distinguishably arrayed. As used herein, the term "distinguishably arrayed" is intended to denote that such gene's (or its fragment or transcript)'s location on the surface is distinct or distinguishable from the locations of other gene(s) that may be bound to the support.

Most preferably, the array will contain gene fragments of hundreds or thousands of human genes. A glass slide containing gene fragments of 9,984 human genes (provided by the Advanced Technology Center of the National Cancer Institute) is preferably employed. Clones and arrays are also available from Incyte Genomics, Palo Alto, CA, and other sources.

For analyzing such microarrays, nucleic acid, most preferably RNA, is isolated from candidate neuroendocrine cells. Any of a wide variety of amplification procedures may be employed. In a preferred embodiment of the invention, a T7-based RNA amplification procedure ins employed, such as that described by Luo, L. *et al.* (1999) ("GENE EXPRESSION PROFILES OF LASER-CAPTURED ADJACENT NEURONAL SUBTYPES" *Nature Medicine 5*:117-22). To facilitate the analysis, the amplified material is preferably labeled, as with a radioactive, fluorescent, chemiluminescent, enzymatic, haptenic, or other label, and incubated with the arrayed gene fragments under conditions suitable for nucleic acid hybridization to occur (see, for example, Schena, M. *et al.* (1995) "QUANTITATIVE MONITORING OF GENE EXPRESSION PATTERNS WITH A COMPLEMENTARY DNA MICROARRAY" *Science 270*:467-70).

The hybridized array are then analyzed for their pattern of hybridization. Detection of hybridization, e.g., detection of the labeled amplified material hybridized to a region of the array, indicates that the gene present at such region was expressed by the candidate cell being analyzed. Most preferably, such analysis will employ an automated scanning device, such as a GenePix 4000A Laser Scanner (Axon Instruments, Inc., Foster City, CA) in conjunction with software for conducting such analysis. The BRB ArrayTools (ver 2.0) is preferred for this purpose (http://linus.nci.nih.gov/BRB-ArrayTools.html).

Having now generally described the invention, the same will be more readily understood through reference to the following examples, which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

Example 1
cDNA Microarray

In order to identify molecular markers of pulmonary neuroendocrine tumors, the gene expression profile of clinical samples from patients with TC, LCNEC, and SCLC is analyzed by cDNA microarrays, preferably as follows:

Tissue Collection And RNA Quality Assessment. Archived, frozen lung tumor tissues are collected from hospitals in the Baltimore, MD metropolitan area over an 11 year period. Tumor tissues are flash-frozen at surgery and stored at –80°C until used. The frozen tumor tissue block is prepared with O.C.T. mount medium and the quality of total RNA of each sample is evaluated by spectrophotometery and gel electrophoresis after phenol/chloroform extraction from one frozen section. Histopathological classification of these tumors is based on the 1999 WHO Classification (Travis, W.D. *et al.* (1999) "HISTOLOGIC TYPING OF LUNG AND PLEURAL TUMORS" (Ed 3). Berlin, Germany, Springer). Two large cell neuroendocrine carcinomas (case 1240 and 1672) are confirmed by demonstrating the neuorendocrine immuno-phenotype with positive NCAM (CD56) staining. **Table 1** summarizes clinical findings in the pulmonary NE tumors.

Table 1
Clinical Features Of 17 Patients With Pulmonary Neuroendocrine Tumors

Histology		Sex		Age		Smoking
		Male	Female	Range	Mean	
TC	(n=11)	7	4	35-68	50	7 (64%)
LCNEC	(n=2)	2	0	59-60	60	2 (100%)
SCLC	(n=4)	3	1	43-75	65	4 (100%)
TOTAL	(n=17)	12	5	35-75	65	13 (100%)

Laser Capture Microdissection Of 17 Neuroendocrine Tumors. Frozen tumor tissue (0.5 x 0.5 x 0.5 cm) are embedded in O.C.T. in a cryomold, and immersed immediately in dry ice-cold 2-methylbutane at –50°C. Sections of frozen tissue (8 mm) are mounted on silane coated glass slides and kept at –80°C until use. The slides are immediately fixed by immersion in 70% ethanol, stained with H&E and air-dried for 10 minutes after xylene treatment.

The PixCell™ LCM system (Arcturus, Moutain View, CA) is used for LCM (Bonner, R.F., *et al.* (1997) "LASER CAPTURE MICRODISSECTION:

MOLECULAR ANALYSIS OF TISSUE," Science 278: 1481,1483). Tumor cells are fused to transfer film by thermal adhesion after laser pulse and were then transferred into tubes containing solution D in the Strategene Micro RNA isolation kit that contains gaunidinium thiocyanate (GTC) and beta-mercaptoethanol.

Figures 1A-1D illustrate carcinoid tumor tissue sections before and after the microdissection. For each specimen, 15 to 18 frozen sections are used to maximize the quantity of RNA. Total RNA is extracted using a Micro RNA isolation kit (Strategene, La Jolla, CA) according to the manufacturer's instructions. Purified total RNA was resuspended in 11 ml of diethyl pyrocarbonate (DEPC), treated water, and used directly for RNA amplification and subjected to cDNA microarray analysis (Schena, M. *et al.* (1995) "QUANTITATIVE MONITORING OF GENE EXPRESSION PATTERNS WITH A COMPLEMENTARY DNA MICROARRAY," Science 270(5235):467-70; DeRisi, J. *et al.* (1996) "USE OF A cDNA MICRO ARRAY TO ANALYSE GENE EXPRESSION PATTERNS IN HUMAN CANCER," Nature Genetics 14:457-60, Lyer, R.P. *et al.* (1999) "MODIFIED OLIGONUCLEOTIDES--SYNTHESIS, PROPERTIES AND APPLICATIONS," Curr. Opin. Mol. Ther. 1:344-358).

RNA Amplification. The RNA amplification procedure used is preferably as described by Luo, L. *et al.* (1999) ("GENE EXPRESSION PROFILES OF LASER-CAPTURED ADJACENT NEURONAL SUBTYPES," Nature Med 5: 117-122). The method relies on attaching a T7 promoter sequence to the oligo(dT) primer. A preferred such sequence for synthesis of the first strand cDNA is **SEQ ID NO.:1**:

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5'   TCTAGTCGAC  GGCCAGTGAA  TTGTAATACG  ACTCACTATA
     GGGCGTTTTT  TTTTTTTTTT  TTTTTT      3'
```

After second strand cDNA synthesis, amplified RNA is generated using T7 RNA polymerase and the double-stranded cDNA molecules as targets for the linear amplification. The T7 promoter sequence is regenerated in subsequent rounds by priming the first strand cDNA synthesis reaction with random hexamers (Amersham Biosciences, Piscataway, NJ). The quality and quantity of amplified

RNA were evaluated spectrophotometricaly and by migration in 2.4% agarose gel electrophoresis, respectively.

Cell Culture. BEAS-2B cell line (Amstad, P. *et al.* (1988) "NEOPLASTIC TRANSFORMATION OF A HUMAN BRONCHIAL EPITHELIAL CELL LINE BY A RECOMBINANT RETROVIRUS ENCODING VIRAL HARVEY RAS," Mol Carcinog. 1988 1:151-60) is cultured in a serum-free medium, LHC-9 (Biofluids, Rockville, MD). Total RNA is isolated from cells with Trizol, followed by phenol/chloroform and isopropanol extraction and purification (Stratagene, La Jolla, CA). Two rounds of amplified RNA are generated from the cell line as described above.

Microarrays Hybridization. cDNA microarrays are performed in duplicate for each sample on glass slides containing 9,984 human genes which were provided by the Advanced Technology Center of the National Cancer Institute. BEAS-2B amplified RNA (8 μg) is labeled with Cy5-dUTP and test samples (4 mg each) are labeled with Cy3-dUTP using Superscript II (Invitrogen, Carlsbad, CA). Briefly, RNA is incubated with Cy3-dUTP (or Cy5-dUTP) (Perkin Elmer Life Sciences, Boston, MA) at 42°C for 1h to synthesize the first strand of cDNA. The reaction is stopped by addition of 5 μl 0.5M EDTA and 10 μl 1N NaOH followed by incubation at 65°C for 60 min. After neutralization, the samples are purified by centrifugation with a Microcon 30 (Millipore Corp., Bedford, MA) to remove unincorporated nucleotides and salts. The Cy3- and Cy5-labeled samples of each pair are combined and heated at 100°C for 2 min. After centrifugation for 10 minutes, the samples are placed onto the center of a glass microarray slide and hybridized at 65°C for 16h. The slides are washed to a final stringency of 0.2 x SSC at room temperature for 2 min prior to analysis.

Image And Statistic Analysis. Hybridized array slides are scanned with a GenePix 4000A Laser Scanner (Axon Instruments, Inc., Foster City, CA). Analysis is performed using BRB ArrayTools (ver 2.0) developed by Drs. Richard Simon and Amy Peng (http://linus.nci.nih.gov/BRB-ArrayTools.html).

Hierarchical clustering was performed on 8,987 clones with log-ratios present in at least 4 samples for each gene.

Example 2
cDNA Microarray Results

The results of the microarray analysis are obtained using Laser Capture Microdissection (LCM) as follows:

Laser Capture Microdissection (LCM) Of Clinical Samples. Use of LCM improves the sample preparation of microarray analysis by avoiding contamination with other cell types. (Emmert-Buck, M.R. *et al.* (1996) "Laser Capture Microdissection," Science 274:998-1001). This selection is particularly desirable for analysis of tumors from lung, prostate, overy, and others (Ornstein, D.K. *et al.* (2000) "PROTEOMIC ANALYSIS OF LASER CAPTURE MICRODISSECTED HUMAN PROSTATE CANCER AND IN VITRO PROSTATE CELL LINES," Electrophoresis 21(11):2235-2242; Mirura, K. *et al.* (2002) "LASER CAPTURE MICRODISSECTION AND MICROARRAY EXPRESSION ANALYSIS OF LUNG ADENOCARCINOMA REVEALS TOBACCO SMOKING- AND PROGNOSIS RELATED MOLECULAR PROFILES," Cancer Res. 62:3244-3250; Ono, K. *et al.* (2000) "IDENTIFICATION BY CDNA MICROARRAY OF GENES INVOLVED IN OVARIAN CARCINOGENESIS," Cancer Res. 60:5007-5011). Tumor cells are selected by LCM from frozen sections. High quality RNA is obtained from these dissected materials. One example of LCM from a TC sample is illustrated in **Figures 1A-1D**.

Microarray Analysis Of Gene Expression Profiles Of Pulmonary Neuroendocrine Tumors. The invention tested the hypothesis that gene expression profiling using cDNA microarray analysis can effectively identify subtypes of pulmonary neuroendocrine tumors classified by light microscopy according to WHO recommendations. Hierarchical clustering of 8,987 human genes, often referred to as unsupervised learning, separated samples into clusters based on overall similarity in gene expression without prior knowledge of sample identity. The hierarchical clustering of genes with statistically significant variance

($p<0.004$) among all tumor samples is displayed in **Figure 2**. After decoding the specimens, it was immediately apparent that clustering based on genome-wide expression divides the tumors into their assigned WHO classification with 100% accuracy. Tumor samples from TC, LCNEC and SCLC clusters with their respective subtype indicating similarities of gene expression shared by these tumors. The length of the branches indicates the relatedness of neuroendocrine tumors. Three distinct groups of tumors can be identified by this display. The sample, which contains features of LCNEC and SCLC clusters between LCNEC and SCLC with a shorter distance to SCLC. Thus, the data support the molecular classification that predicted morphological classification of human pulmonary neuroendocrine tumors. The data indicates that WHO proposed morphological classification of pulmonary neuroendocrine tumors correspond to a significant similarity of their molecular profiles.

The Class Comparison Tool is used to select genes differentially expressed among each tumor type at an assigned statistical significance level. The F-test, which measures levels of variance in gene expression among each sample, is used to compare the defined classes of tumors using BRB ArrayTool. This analysis results in the identification of a set of 198 genes that have statistically significant variance ($p<0.004$, **Table 2**). Having selected these 198 genes, another hierarchical clustering can be created by enforcing the classification of 17 tumors (**Figure 3**). The results show that the tumors cluster together in 3 groups in complete agreement with the pre-assigned morphological classification. Samples from LCNEC cluster closer to TC than to SCLC and the tumor that contained features of small and large neuroendocrine cells clustered with SCLC which confirms the molecular relatedness identified by genome-wide expression in clinical behavior of these tumors. The results show that most of the 198 selected genes could be assigned to major functional groups that have been previously implicated in cancer development (**Table 3**). In particular, decreased expression of genes that oppose cell survival pathway, such as BCL2 antagonist-killer, BAK1, and caspase 4, are common in all 3 types of neuroendocrine tumors, whereas TC

and LCNEC have an additional >2.5-fold decrease in expression of BAS and TNF receptor-interacting kinase, RIPK1. These featuresindicate that these tumors lack opposing effects on BCL2, as contrasted to overexpression of BCL2, which leads to survival advantage in certain types of lymphomas (Cleary, M.L. *et al.* (1986) "CLONING AND STRUCTURAL ANALYSIS OF CDNAS FOR BCL-2 AND A HYBRID BCL-2/IMMUNOGLOBULIN TRANSCRIPT RESULTING FROM THE T(14;18) TRANSLOCATION," Cell. 47(1):19-28) **(Figure 2).**

Table 2

Genes Having Statistically Significant Variance in Expression in Neuroendocrine Tumor Cells

Unique ID No.	Description	Gene Symbol (Map)	Incyte Clone ID No.	UG Cluster
Cluster #1				
166807	glutamate receptor, ionotropic, AMPA 2 Neuronal Marker, TM Receptor	GRIA2 [4q32-q33]	IncytePD 1505977	Hs.89582
159877	carboxypeptidase E Secreted Lys Neuronal M	CPE [4q32 3]	IncytePD,2153373	Hs.75360
161598	origin recognition complex, subunit 4 (yeast homolog)-like	ORC4L [2q22-q23]	IncytePD 2728840	Hs 55055
167158	complement component 5 Infl Resp. VP Extracellular	C5 [9q32-q34]	IncytePD·1712663	Hs 1281
Cluster #2				
167153	gamma-glutamyl hydrolase (conjugase, folylpolygammaglutamyl hydrolase) Protease, Lys	GGH [8q12 1]	IncytePD·1997967	Hs 78619
160605	P311 protein Invasion marker, Adhesion Plaques	P311 [5q21 3]	IncytePD 1555545	Hs 142827
169429	nuclear receptor subfamily 3, group C, member 1 Glucocort Rec/TF	NR3C1 [5q31]	IncytePD·629077	Hs 75772
165192	synaptojanin 2 IP3 5-Phosphatase	SYNJ2 [6q25-26]	IncytePD.3954785	Hs 61289
165784	adducin 3 (gamma) Cytoskel	ADD3 [10q24 2-q24 3]	IncytePD·1481225	Hs 324470
163031	KIAA0751 gene product	KIAA0751 [8q23 1]	IncytePD 2369544	Hs.153610
166328	proteasome (prosome, macropain) 26S subunit, ATPase, 6 Proteasome	PSMC6 [12q15]	IncytePD·1488021	Hs 79357
168061	formyltetrahydrofolate dehydrogenase NADPH Sx, Folic Acid One-carbon meth	FTHFD [3q21 3]	IncytePD·2104145	Hs 9520
168141	diacylglycerol kinase, gamma (90kD)	DGKG [3q27-q28]	IncytePD.2568547	Hs 89462
165076	PI-3-kinase-related kinase SMG-1 RNA Surveillance	SMG1 [16p12.3]	IncytePD 4253663	Hs 110613
167103	TAF2 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 150 kD TATA Box TF	TAF2 [8q24 12]	IncytePD 998069	Hs 122752

Table 2

Genes Having Statistically Significant Variance in Expression in Neuroendocrine Tumor Cells

Unique ID No.	Description	Gene Symbol (Map)	Incyte Clone ID No.	UG Cluster
169391	eukaryotic translation initiation factor 2, subunit 1 (alpha, 35kD) polysome	EIF2S1 [14q23 3]	IncytePD 1224219	Hs 151777
166789	zinc finger protein 202 Transcriptional Repressor	ZNF202 [11q23 3]	IncytePD 1997937	Hs 9443
167316	solute carrier family 24 (sodium/potassium/calcium exchanger), member 1 Sodium/potassium/calcium exchanger	SLC24A1 [15q22]	IncytePD·2200079	Hs 173092
168700	formyl peptide receptor-like 1 Integram Membr/Migration/Expressed in Lung	FPRL1 [19q13 3-q13 4]	IncytePD.523635	Hs 99855
165576	interleukin 6 signal transducer (gp130, oncostatin M receptor)	IL6ST [5q11]	IncytePD.2172334	Hs.82065
168276	integrin, beta-like 1 (with EGF-like repeat domains)	ITGBL1 [13q33]	IncytePD 1258790	Hs 82582
169180	interleukin 8 receptor, beta	IL8RB [2q35]	IncytePD 561992	Hs 846
160957	protein kinase, AMP-activated, alpha 2 catalytic subunit	PRKAA2 [1p31]	IncytePD.2507648	Hs 2329
160617	colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)	CSF2RB [22q13 1]	IncytePD.1561352	Hs 285401
160429	PTK6 protein tyrosine kinase 6 Non-Receptor, Sensitizes to EGF	PTK6 [20q13 3]	IncytePD·3255437	Hs 51133
160237	nuclear protein, ataxia-telangiectasia locus Osteogenesis Imperfecta	NPAT [11q22-q23]	IncytePD 2308525	Hs 89385
167125	tumor necrosis factor receptor superfamily, member 6	TNFRSF6 [10q24 1]	IncytePD 2205246	Hs 82359
164652	platelet-derived growth factor receptor, beta polypeptide	PDGFRB [5q31-q32]	IncytePD.1821971	Hs.76144
161117	ATP-binding cassette, sub-family G (WHITE), member 2 Multidrug Resistance	ABCG2 [4q22]	IncytePD.1501080	Hs.194720
161896	collagen, type XV, alpha 1	COL15A1 [9q21-q22]	IncytePD.4287342	Hs.83164
159813	protein tyrosine phosphatase, non-receptor type 12 PEST Dom; p-c-Abl, Ctx Cell shape/motility	PTPN12 [7q11.23]	IncytePD:1382374	Hs 62
164573	cyclin D binding Myb-like transcription factor 1 Not reported to be Expressed in Lung	DMTF1 [7q21]	IncytePD 1637517	Hs.5671
169384	solute carrier family 22 (organic cation transporter), member 1-like antisense Organic-Cation Transporter-Like 2-Antisense	SLC22A1LS [11p15 5]	IncytePD 3842669	Hs 300076
165393	ESTs, Weakly similar to 2109260A B cell growth factor [H sapiens]		IncytePD 3202075	Hs 351699
168169	3-oxoacid CoA transferase mitochondrial matrix coenzyme A from succinyl-CoA to acetoacetate	OXCT [5p13]	IncytePD 1685342	Hs 177584
165617	prolactin receptor	PRLR [5p14-p13]	IncytePD.3427560	Hs.1906
169432	interleukin 13 receptor, alpha 2	IL13RA2 [Xq13.1-q28]	IncytePD.3360476	Hs.25954

Table 2

Genes Having Statistically Significant Variance in Expression in Neuroendocrine Tumor Cells

Unique ID No.	Description	Gene Symbol (Map)	Incyte Clone ID No.	UG Cluster
166812	myelin protein zero-like 1 extracellular membrane face	MPZL1 [1q23 2]	IncytePD 2057323	Hs 287832
168428	runt-related transcription factor 3	RUNX3 [1p36]	IncytePD·885297	Hs 170019
167180	S100 calcium-binding protein A4 (calcium protein, calvasculin, metastasin, murine placental homolog) cell cycle progression, Associated with mets	S100A4 [1q21]	IncytePD.1222317	Hs 81256
161533	cleavage stimulation factor, 3' pre-RNA, subunit 2, 64kD RNA processing/modification	CSTF2 [Xq21 33]	IncytePD 4016254	Hs 693
165588	small nuclear RNA activating complex, polypeptide 4, 190kD	SNAPC4 [9q34 3]	IncytePD.2224902	Hs 113265
164799	epithelial membrane protein 3 cell-cell interactions. Promotes Apoptosis	EMP3 [19q13 3]	IncytePD 780992	Hs 9999
161709	hypothetical protein FLJ11560	FLJ11560 [9p12]	IncytePD 1990361	Hs 301696
164868	guanylate binding protein 2, interferon-inducible GTP-ase	GBP2 [1pter-p13 2]	IncytePD.1610993	Hs.171862
160233	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 3 Cell growth, P-histones, Transcription	DYRK3 [1q32]	IncytePD 614679	Hs.38018
165400	hypothetical brain protein my040 Overexp Lung neuroendocrine tumors	MY040 [7q35-q36]	IncytePD·2048144	Hs 124854
165957	pancreatic lipase-related protein 2 Hydrolyse	PNLIPRP2 [10q26.12]	IncytePD·885032	Hs 143113
160054	GTP-binding protein homologous to Saccharomyces cerevisiae SEC4 Sec vesicles SC	SEC4L [17q25 3]	IncytePD.1824556	Hs 302498
162475	cancer/testis antigen 2 melanomas, non-small-cell lung carcinomas, bladder, Prostate, H/N	CTAG2 [Xq28]	IncytePD·849425	Hs 87225
169182	testis-specific ankyrin motif containing protein	LOC56311 [7q31]	IncytePD 2013272	Hs 73073
162912	nectin 3 PVRL1, may be a membrane glycoprotein	DKFZP566B084 [3q13]	IncytePD 2680168	Hs 21201
163475	hypothetical protein 7q22 1 102-113	FLJ20485 [7q22 1]	IncytePD.2299818	Hs 98806
164927	heterogeneous nuclear ribonucleoprotein A0 RNA processing/modification	HNRPA0 [5q31]	IncytePD·637639	Hs 77492
160630	homeo box D9 RNA processing/modification	HOXD9 [2q31-q37]	IncytePD·2956581	Hs.236646
160367	v-jun avian sarcoma virus 17 oncogene homolog Associated with transl in Tumors	JUN [1p32-p31]	IncytePD·1969563	Hs.78465
163762	ESTs ~	[17]	IncytePD 1743234	Hs.120854
162247	very large G protein-coupled receptor 1 transports Ca2+ during excitation-contraction	VLGR1 [5q13]	IncytePD 942207	Hs.153692
167219	pumilio (Drosophila) homolog 1	PUM1 [1p35.2]	IncytePD 3333130	Hs 153834

Table 2

Genes Having Statistically Significant Variance in Expression in Neuroendocrine Tumor Cells

Unique ID No.	Description	Gene Symbol (Map)	Incyte Clone ID No.	UG Cluster
Cluster #3				
165171	keratin 18	KRT18 [12q13]	IncytePD·1435374	Hs 65114
165052	CDC20 (cell division cycle 20, S cerevisiae, homolog) Cell cycle, microtubule-dependent processes	CDC20 [9q13-q21]	IncytePD:2469592	Hs 82906
167948	pim-1 oncogene S T kinase Hematop Cells	PIM1 [6p21 2]	IncytePD 2679117	Hs 81170
161954	ATPase, H+ transporting, lysosomal (vacuolar proton pump) 21kD Vacuolar H Transporter	ATP6F [1p32 3]	IncytePD·5017148	Hs 7476
162391	polymerase (DNA directed), epsilon 3 (p17 subunit) DNA Replication	POLE3 [9q33]	IncytePD·961082	Hs 108112
166635	keratin 5 (epidermolysis bullosa simplex, Dowling-Meara/Kobner/Weber-Cockayne types)	KRT5 [12q12-q13]	IncytePD 3432534	Hs.195850
160035	flap structure-specific endonuclease 1 DNA Repair/UV rad protection	FEN1 [11q12]	IncytePD.2050085	Hs 4756
161774	calcium and integrin binding protein (DNA-dependent protein kinase interacting protein)	SIP2-28 [15q25 3-q26]	IncytePD 4626895	Hs 10803
162207	membrane protein of cholinergic synaptic vesicles vesicular transport	VATI [17q21]	IncytePD.2060308	Hs.157236
161163	guanylate kinase 1 Sx GTP/GMP	GUK1 [1q32-q41]	IncytePD.2506427	Hs 3764
161223	CD27-binding (Siva) protein tumor necrosis receptor (TFNR) superfamily	SIVA [22]	IncytePD 2356635	Hs.112058
161211	capping protein (actin filament), gelsolin-like	CAPG [2cen-q24]	IncytePD 2508570	Hs.82422
161948	claudin 11 (oligodendrocyte transmembrane protein)	CLDN11 [3q26.2-q26 3]	IncytePD.4144001	Hs 31595
161391	interleukin 17F	IL17F [6p12]	IncytePD.1610083	Hs 272295
162571	phosphofructokinase, liver	PFKL [21q22.3]	IncytePD·885601	Hs 155455
164504	cathepsin C Lys Prot Degr	CTSC [11q14 1-q14 3]	IncytePD 1822716	Hs.10029
160565	aminoacylase 1 L-aa Sx salvage path	ACY1 [3p21 1]	IncytePD 1812955	Hs 334707
169551	glycogen synthase kinase 3 beta target of Akt, Ilk1, Reg jun, myb, etc	GSK3B [3q13 3]	IncytePD·2057908	Hs 78802
166914	methyltransferase-like 1 S-adenosylmethionine-binding mo	METTL1 [12q13]	IncytePD.1603584	Hs 42957
167738	cytochrome P450, subfamily XXVIIB (25-hydroxyvitamin D-1-alpha-hydroxylase), polypeptide 1 drug metabolism and synthesis of cholesterol, steroids	CYP27B1 [12q13 1-q13 3]	IncytePD.1749727	Hs 199270
160938	GrpE-like protein cochaperone cooperates with mitochondrial hsp70 r	HMGE [4p16]	IncytePD 2074154	Hs 151903
162734	wingless-type MMTV integration site family, member 7A Regulates Steroid responses	WNT7A [3p25]	IncytePD 2622566	Hs 72290

Table 2

Genes Having Statistically Significant Variance in Expression in Neuroendocrine Tumor Cells

Unique ID No.	Description	Gene Symbol (Map)	Incyte Clone ID No.	UG Cluster
165813	caspase 4, apoptosis-related cysteine protease	CASP4 [11q22 2-q22 3]	IncytePD 2304121	Hs 74122
159898	pituitary tumor-transforming 1	PTTG1 [5q35 1]	IncytePD 1748705	Hs 252587
161244	ADP-ribosylation factor 4-like GTP-binding proteins. ARF4L is c	ARF4L [17q12-q21]	IncytePD 2852403	Hs.183153
160715	cell division cycle 34	CDC34 [19p13 3]	IncytePD.1857493	Hs 76932
163787	pyrroline-5-carboxylate reductase 1 Proline Sx	PYCR1 [17q24]	IncytePD:1702266	Hs 79217
160127	phosphoglycerate mutase 1 (brain)	PGAM1 [10q25 3]	IncytePD.3032691	Hs.181013
160323	5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase Purine BioSx	ATIC [2q35]	IncytePD.2056149	Hs.90280
164850	interleukin-1 receptor-associated kinase 1	IRAK1 [Xq28]	IncytePD:1872067	Hs 182018
165583	7-dehydrocholesterol reductase	DHCR7 [11q13.2-q13 5]	IncytePD:3518380	Hs 11806
165039	thymidine kinase 1, soluble two forms have been identified in animal cells	TK1 [17q23 2-q25 3]	IncytePD 2055926	Hs.105097
167984	cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)	CDKN2A [9p21	IncytePD.2740235	Hs 1174
167223	guanine nucleotide binding protein (G protein), beta polypeptide 1 Ras GTPase, Contains 7 wd repeats	GNB1 [1p36 21-36 33]	IncytePD 3562795	Hs 215595
167931	cleavage stimulation factor, 3' pre-RNA, subunit 1, 50kD RNA, transducin-like repeats	CSTF1 [20q13 2]	IncytePD.1635008	Hs 172865
163690	hexabrachion (tenascin C, cytotactin)	HXB [9q33]	IncytePD.1453450	Hs.289114
161955	contactin 2 (axonal)	CNTN2 [1q32 1]	IncytePD.4014715	Hs.2998
160275	structure specific recognition protein 1	SSRP1 [11q12	IncytePD:2055773	Hs 79162
168110	TAF12 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 20 kD	TAF12 [1p35 1]	IncytePD 1297269	Hs 82037
160102	protein disulfide isomerase related protein (calcium-binding protein, intestinal-related) Sevretion, ER	ERP70 [10]	IncytePD 1824957	Hs 93659
167116	nucleoside phosphorylase adenosine deaminase (ADA) serves a key role in purine catabolism, Def=SCID	NP [14q13 1]	IncytePD:2453436	Hs 75514
160802	prohibitin Tumor suppressor, Blocks DNA Sx; Breast CA	PHB [17q21]	IncytePD.1625169	Hs.75323
161643	ADP-ribosylation factor-like 7 GTP-binding protein	ARL7 [2q37 2]	IncytePD 3115514	Hs 111554
162343	LIM domain kinase 2 Rho-induced reorganization of the actin cytoskeleton	LIMK2 [22q12 2]	IncytePD 958513	Hs 278027
162727	protein tyrosine kinase 9-like (A6-related protein)	PTK9L [3p21 1]	IncytePD:3999291	Hs.6780

Table 2

Genes Having Statistically Significant Variance in Expression in Neuroendocrine Tumor Cells

Unique ID No.	Description	Gene Symbol (Map)	Incyte Clone ID No.	UG Cluster
160262	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 28 probable atp-binding rna helicase	DDX28 [16q22.1]	IncytePD 2663948	Hs 155049
165790	surfeit 1 Mit Resp Enz	SURF1 [9q33-q34]	IncytePD.1921567	Hs 3196
168638	histone deacetylase 7A	HDAC7A [12q13.1]	IncytePD:1968721	Hs 275438
168079	epithelial membrane protein 1 cell-cell interactions Promotes Apoptosis	EMP1 [12p12 3]	IncytePD·1624024	Hs.79368
160999	Rho-specific guanine nucleotide exchange factor p114 cell growth and motility; Dbl, PH dom	P114-RHO-GEF [19p13 3]	IncytePD·1734113	Hs 6150
161790	KIAA0469 gene product	KIAA0469 [1p36.23]	IncytePD 2674277	Hs.7764
169691	ubiquitin carrier protein E2 enzyme activity	E2-EPF [17p12-p11]	IncytePD 2057823	Hs 174070
163682	diptheria toxin resistance protein required for diphthamide biosynthesis (Saccharomyces)-like 2	DPH2L2 [1p34]	IncytePD 1810821	Hs 324830
168266	proteasome (prosome, macropain) activator subunit 3 (PA28 gamma; Ki)	PSME3 [17q12-q21]	IncytePD·1308112	Hs 152978
161374	polymerase (DNA-directed), alpha (70kD) RNA Processing	POLA2 [11q13 1]	IncytePD.3179113	Hs 81942
164646	galactose-4-epimerase, UDP-Rate-lim for Sx glycoproteins and glycolipids	GALE [1p36-p35]	IncytePD.1807294	Hs 76057
162150	apolipoprotein L	APOL1 [22q13 1]	IncytePD 2056987	Hs.114309
164206	type I transmembrane protein Fn14 similar to murine Fgfrp2	FN14 [16p13.3]	IncytePD 1402615	Hs 10086
162623	BCL2-antagonist/killer 1	BAK1 [6p21 3]	IncytePD·2055687	Hs.93213
162244	Rho GDP dissociation inhibitor (GDI) alpha	ARHGDIA [17q25 3]	IncytePD 2055640	Hs.159161
164586	inosine triphosphatase (nucleoside triphosphate pyrophosphatase) Ins Phos phosphatase	ITPA [20p]	IncytePD:1931265	Hs.6817
165483	PDGFA associated protein 1 Enhances PDGFA	PDAP1 [7q22 1]	IncytePD.3032825	Hs 278426
166195	adenine phosphoribosyltransferase Sx AMP purine/pyrimidine Met	APRT [16q24]	IncytePD.2751387	Hs 28914
166960	Apg12 (autophagy 12, S cerevisiae)-like	APG12L [5q21-q22]	IncytePD.2058537	Hs 264482
167505	thiosulfate sulfurtransferase (rhodanese) Mitoch detox cyanide	TST [22q13 1]	IncytePD.1988239	Hs 351863
168642	suppression of tumorigenicity 14 (colon carcinoma, matriptase, epithin) Protease ECM	ST14 [11q24-q25]	IncytePD 478960	Hs 56937
167170	GS2 gene	DXS1283E [Xp22 3]	IncytePD 1567995	Hs 264
161754	actin, gamma 2, smooth muscle, enteric	ACTG2 [2p13 1]	IncytePD·3381870	Hs.78045
166010	receptor (TNFRSF)-interacting serine-threonine kinase 1	RIPK1 [6p25 3]	IncytePD 2180031	Hs 296327
161794	secretory carrier membrane protein	SCAMP2	IncytePD.3123858	Hs 238030

Table 2

Genes Having Statistically Significant Variance in Expression in Neuroendocrine Tumor Cells

Unique ID No.	Description	Gene Symbol (Map)	Incyte Clone ID No.	UG Cluster
	2 Vesic Traff, Secretpry path	[15q23-q25]		
167591	catechol-O-methyltransferase Sx dopamine, epinephrine, and norepinephrine	COMT [22q11 21]	IncytePD 605019	Hs.240013
162587	polymerase (RNA) II (DNA directed) polypeptide D RNA Processing	POLR2D [2q21]	IncytePD.696002	Hs.194638
169071	capping protein (actin filament) muscle Z-line, beta	CAPZB [1p36 1]	IncytePD.1853163	Hs.333417
160467	polymerase (DNA directed), delta 2, regulatory subunit (50kD) RNA Processing	POLD2 [7p13]	IncytePD 2056172	Hs.74598
162178	C2f protein	C2F [12p13]	IncytePD 5096975	Hs.12045
167706	GDP-mannose pyrophosphorylase B N-linked oligosaccharides	GMPPB [3p21 31]	IncytePD 1486983	Hs.28077
160803	phenylalanine-tRNA synthetase-like Reg. in tumors and cell cycle	FARSL [19p13.2]	IncytePD 1808260	Hs.23111
169254	polymerase (DNA directed), mu RNA Processing	POLM [7p13]	IncytePD 771715	Hs.46984
167351	myosin-binding protein H	MYBPH [1q32 1]	IncytePD 3010959	Hs.927
163276	ESTs, Weakly similar to I37356 epithelial microtubule-associated protein, 115K [H sapiens]	[7]	IncytePD.2383065	Hs.25892
167135	excision repair cross-complementing rodent repair deficiency, complementation group 1 (includes overlapping antisense sequence)	ERCC1 [19q13.2-q13 3]	IncytePD.2054529	Hs.59544
160478	G5b protein	G5B [6p21 3]	IncytePD 1942845	Hs 73527
162631	transcriptional adaptor 3 (ADA3, yeast homolog)-like (PCAF histone acetylase complex) PCAF histone acetilase complex	TADA3L [3p25 2]	IncytePD 3990209	Hs.158196
163921	glucosamine-6-phosphate isomerase Hydrolase	GNPI [5q21]	IncytePD 1653911	Hs 278500
160098	mitochondrial ribosomal protein L49	MRPL49 [11q13]	IncytePD 1755793	Hs.75859
161058	multiple endocrine neoplasia I	MEN1 [11q13]	IncytePD 1693847	Hs 24297
160038	BCL2-antagonist of cell death	BAD [11q13.1]	IncytePD 3967780	Hs 76366
162220	FK506-binding protein 1A (12kD) Interacts with TGF beta	FKBP1A [20p13]	IncytePD:4059193	Hs.349972
161026	Xq28, 2000bp sequence contg. ORF 3' eDNA Repair xonuclease activity	HSXQ28ORF [Xq28]	IncytePD 1669254	Hs 6487
167607	heat shock protein 75 HSP90 fam, Binds to TNFR	TRAP1 [16p13.3]	IncytePD:1960722	Hs 182366
167713	likely ortholog of maternal embryonic leucine zipper kinase regulation of fatty acid synthesis	KIAA0175 [9p11.2]	IncytePD 3805046	Hs 184339
165648	dual specificity phosphatase 4 negatively regulate MAPK Anti-oncogene	DUSP4 [8p12-p11]	IncytePD 740878	Hs 2359
161574	frequently rearranged in advanced	FRAT2	IncytePD 3871545	Hs.140720

Table 2

Genes Having Statistically Significant Variance in Expression in Neuroendocrine Tumor Cells

Unique ID No.	Description	Gene Symbol (Map)	Incyte Clone ID No.	UG Cluster
	T-cell lymphomas 2 prevent gsk-3-dependent phosphorylation	[10q23-q24 1]		
161650	KIAA0415 gene product	KIAA0415 [7p22 2]	IncytePD·2798872	Hs.229950
168386	nucleolar and coiled-body phosphprotein 1	NOLC1 [10]	IncytePD·1431819	Hs.75337
159906	H2A histone family, member X	H2AFX [11q23 2-q23 3]	IncytePD.1704168	Hs 147097
167906	RAE1 (RNA export 1, S pombe) homolog RNA export from the N	RAE1 [20q13 31]	IncytePD 2914719	Hs.196209
160486	deltex (Drosophila) homolog 2 collagen type III	DTX2 [7q11 23]	IncytePD 1691161	Hs 89135
160678	v-maf musculoaponeurotic fibrosarcoma (avian) oncogene family, protein G transcriptional regulator	MAFG [17q25]	IncytePD 2956906	Hs.252229
159889	fusion, derived from t(12,16) malignant liposarcoma DNA Sx atp-independent annealing of complementary single- stranded dnas	FUS [16p11 2]	IncytePD 3038508	Hs.99969
167553	ligase I, DNA, ATP-dependent DNA excision repair process	LIG1 [19q13 2-q13 3]	IncytePD 1841920	Hs.1770
163824	uracil-DNA glycosylase DNA Base-excision repair	UNG [12q23-q24 1]	IncytePD·1405652	Hs 78853
161012	GCN1 (general control of amino-acid synthesis 1, yeast)-like 1	GCN1L1 [12q24.2]	IncytePD 1699149	Hs.75354
162006	regenerating islet-derived 1 beta (pancreatic stone protein, pancreatic thread protein) brain and pancreas regeneration	REG1B [2p12]	IncytePD.2374294	Hs 4158
161454	serine protease inhibitor, Kunitz type 1 Secreted S/Protease; proteolytic activation of HGF	SPINT1 [15q13.3]	IncytePD·2722572	Hs 233950
162510	calcium/calmodulin-dependent protein kinase kinase 2, beta S/T Protein kinase	CAMKK2 [12]	IncytePD 557451	Hs 108708
163308	Bloom syndrome DNA Repair	BLM [15q26 1]	IncytePD 2923082	Hs 36820
160242	RNA, U transporter 1	RNUT1	IncytePD 1562658	Hs 21577
164106	glutamate rich WD repeat protein GRWD RNA stability	GRWD [19q13.33]	IncytePD 1561867	Hs 218842
165799	MAD (mothers against decapentaplegic, Drosophila) homolog 3 TF, activated by tgf-beta	MADH3 [15q21-q22]	IncytePD 1858365	Hs.211578
166574	small nuclear RNA activating complex, polypeptide 2, 45kD RNA Processing	SNAPC2 [19p13 3-p13 2]	IncytePD 1445203	Hs 78403
160441	lymphotoxin beta receptor (TNFR superfamily, member 3) TNF family of receptors	LTBR [12p13]	IncytePD·899102	Hs 1116
168453	transforming, acidic coiled-coil containing protein 3 Upregulated in Tumors	TACC3 [4p16.3]	IncytePD.2056642	Hs.104019
164244	proteasome (prosome, macropain) 26S subunit, ATPase, 4	PSMC4 [19q13 11-q13 13]	IncytePD 2806778	Hs 211594

Table 2

Genes Having Statistically Significant Variance in Expression in Neuroendocrine Tumor Cells

Unique ID No.	Description	Gene Symbol (Map)	Incyte Clone ID No.	UG Cluster
169564	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 2 TF	SMARCD2 [17q23-q24]	IncytePD 1890919	Hs 250581
161178	basigin (OK blood group) Induces MMTP, p-regulated in gliomas	BSG [19p13.3]	IncytePD·2182907	Hs 74631
165614	junction plakoglobin	JUP [17q21]	IncytePD 820580	Hs 2340
168987	HMT1 (hnRNP methyltransferase, S cerevisiae)-like 2 Protein methylation	HRMT1L2 [19q13 3]	IncytePD:2888814	Hs.20521
167987	ectonucleoside triphosphate diphosphohydrolase 1 ATP hydrolysis, Plt aggregation	ENTPD1 [10q24]	IncytePD:1672749	Hs.205353
163726	complement component 3	C3 [19p13 3-p13 2]	IncytePD.1513989	Hs 284394
164642	tyrosyl-tRNA synthetase	YARS [1p34 3]	IncytePD.1559756	Hs 239307
160303	Ets2 repressor factor	ERF [19q13]	IncytePD:2057547	Hs 333069
161635	G protein-coupled receptor	TYMSTR [3p21]	IncytePD:2610374	Hs 34526
159859	nuclear autoantigen wd REPEAT PROTEIN	GS2NA [14q13-q21]	IncytePD 1339241	Hs.183105
161051	MAP/microtubule affinity-regulating kinase 3 S/T Protein kinase	MARK3 [14q32.3]	IncytePD 2395018	Hs.172766
161835	peroxisome biogenesis factor 10	PEX10 [1p36.11-1p36.33]	IncytePD 3115936	Hs 247220
165571	annexin A3 calcium-dependent phospholipid-binding	ANXA3 [4q13-q22]	IncytePD.1920650	Hs.1378
164286	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon	NFKBIE [6p21.1]	IncytePD 2748942	Hs.91640
165786	hyaluronoglucosaminidase 2 Degrades glycosaminoglycans of the extracellular matrix	HYAL2 [3p21.3]	IncytePD:1240748	Hs.76873
161620	H4 histone family, member E	H4FE [6p22-p21 3]	IncytePD·3728255	Hs.278483
168302	Tax interaction protein 1 1 pdz/dhr domain	TIP-1 [17p13]	IncytePD·1997792	Hs 12956
160887	pescadillo (zebrafish) homolog 1, containing BRCT domain embrional dev	PES1 [22q12 1]	IncytePD·2758740	Hs.13501
162419	RAE1 (RNA export 1, S.pombe) homolog	RAE1 [20q13 31]	IncytePD·588157	Hs 196209
169625	replication factor C (activator 1) 4 (37kD) DNA Sx/Repair	RFC4 [3q27]	IncytePD 1773638	Hs 35120
163425	transcription elongation factor A (SII), 2	TCEA2 [20]	IncytePD 818568	Hs 80598
166359	tubulin, beta polypeptide Testis-specific	TUBB [6p21.3]	IncytePD.3334367	Hs 336780
161947	translocase of inner mitochondrial membrane 17 homolog B (yeast) Integral Mitoch Expr. In Neuroendocr Lung CA	TIM17B [Xp11.23]	IncytePD·1727491	Hs 19105
162236	KIAA0670 protein/acinus	KIAA0670 [14q11 1]	IncytePD.1968610	Hs 227133

Table 2

Genes Having Statistically Significant Variance in Expression in Neuroendocrine Tumor Cells

Unique ID No.	Description	Gene Symbol (Map)	Incyte Clone ID No.	UG Cluster
168426	glioma pathogenesis-related protein	RTVP1 [12q15]	IncytePD 477045	Hs.64639

Characteristics Of The Gene Expression Patterns In Pulmonary Neuroendocrine Tumors. The present invention permits investigation of whether expression of genes significantly altered in neuroendocrine tumors correlates with clinical behavior of these tumors. The results show that most of 198 selected genes could be assigned to major functional groups that have been previously implicated in cancer development (**Table 3**). In particular, decreased expression of genes that oppose cell survival pathway, such as BCL2 antagonist-killer, BAK1, and caspase 4, are common in all 3 types of neuroendocrine tumors, whereas TC and LCNEC have an additional >2.5-fold decrease in expression of BAD and TNF receptor-interacting kinase, RIPK1. These features indicate that these tumors lack opposing effects on BCL2, as contrasted to overexpression of BCL2, which leads to survival advantage in certain types of lymphomas (Cleary, M.L. *et al.* (1986) "CLONING AND STRUCTURAL ANALYSIS OF CDNAS FOR BCL-2 AND A HYBRID BCL-2/IMMUNOGLOBULIN TRANSCRIPT RESULTING FROM THE T(14;18) TRANSLOCATION," Cell. 47(1):19-28).

Genes involved in regulation of cell-cell and extracellular matrix interactions, claudin 11 (CLDN11), contractin-2, (CNTN2), keratin 5 and 18 (KRT 5 and 18), calcium and integrin binding protein (SIP2-28), and junction plakoglobulin (JUP) are also suppressed in TC and LCNEC tumors, and, to a lesser degree, in SCLC. The dominant group of genes is involved in transcriptional regulation and DNA synthesis and repair. Decrease in expression of Bloom (BLM) is shared by TC and LCNEC, whereas DNA excision repair (ERCC1) and DNA ligase-1 (LIG) are suppressed in all tumor types. Other groups of genes manifesting decreased expression in all tumors are genes involved in cell cycle control (CDC34, p16/CDK inhibitor 2A), suppressor of MAPK pathway (dual specificity phosphatase, DUSP4), antioncogenes, such as epithin (ST14), and

prohibitin, (PHB). Decreased expression of genes involved in microtubular assembly, beta tubulin polypepetide B (TUBB) in conjunction with overexpression of ATP-binding cassette protein (ABCG2) and gamma glutamyl hydrolase (GGH), could confer well-known resistance of these tumors to chemotherapy, specifically to taxol-related drugs. Decreased expression of genes associated with the ubiquitin pathway, such as proteasome subunit 26S (PSMC4), and proteasome activator subunit 3 (PSME3), correlates with potential resistance to newly developed proteasome inhibitors. The decrease in expression of these genes can affect NFkB activity, drug resistance and other functions in these tumors.

Only a fraction of genes identified herein is significantly over-expressed. Expression of a neuroendocrine peptide processing enzyme, carboxypeptidase E (CPE), inotropic glutamate receptor (GRIA2) and a complement component 5 are increased 4-6-fold in TC. In addition, TC has a modest increase in expression of the IL8 receptor B, IL8RB (1.61-fold), and that of the interleukin 6 signal transducer chain common to several interleukin receptors, gp130 (Oncostatin M, IL6ST), which is elevated at a mean of 1.34-fold in the 11 samples from TC. In contrast, LCNEC, have over 20 genes whose expression is above 1.9-fold or higher (**Figures 4A** and **4B**). These gene products are increased specifically in LCNEC and included colony stimulating factor receptor (CSF2R), IL 13 receptor (IL13RA2), IL-8 receptor beta (IL8RB) as well as the IL 6 signal transducer, gp130 (Oncostatin M, IL6ST) and gamma-glutamyl hydrolase (GGH), which has been associated with drug resistance. In addition, LCNEC have a six-fold over-expression of a neuronal marker, P311, recently identified as a marker of aggressive gliomas. P311 may have a role in defining a metastatic/invasive potential in LCNEC. In contrast to LCNEC, analysis of SCLC shows only modest increase in 25 genes, none of which exceeded 1.5-fold increase. The lack of detection of over-expressed genes in SCLC reported herein could reflect a qualitative change in oncogenic mutations, such as $p21^{ras}$, p53 and others which are found in SCLC (Wistuba, I.I. *et al.* (2001) "MOLECULAR GENETICS OF SMALL CELL LUNG CARCINOMA," Semin. Oncol. 28: 3-13) or due to limited number of samples used.

Table 3

Unique ID No. of Gene		Expression of Genes in Large Cell (LC), Small Cell (SC) and Typical Carcinoma (TC) Cells		
Gene Family	(LOH)	LC	SC	TC
Apoptosis				
167125	Yes	3 23	0 88	1 36
162623	Yes	0 23	0 51	0 13
160038	Yes	0 47	1.04	0.32
165813		0.59	0.75	0 28
168079		0 46	0.93	0 25
164799	Yes	1 2	0.73	0 64
160441		0 37	0 49	0 18
161223		0 2	0 71	0 11
166010		0 45	0.99	0.28
167607		0.4	0.81	0 23
166960		0 17	0 37	0 09
Cell-Cell And ECM Interactions				
168700	Yes	1 91	0 82	1 69
168276		1 61	0 63	1 21
162912		0.82	0 7	1 27
161896		2.12	0 75	1 04
159813		1 99	0 83	1.22
166812		0 93	0 78	0 78
165171		0.3	0 16	0 05
166635		0.18	0 63	0 11
161774	Yes	0 2	0 57	0.11
161211		0 27	0 64	0 12
161948		0 19	0.56	0.09
162734		0 73	1 01	0.32
163690		0 42	0.82	0 23
161955		0.17	0.38	0 09
164208		0 26	0 53	0 11
168642		0.55	0 96	0.3
160486		0 37	0.72	0.19
161178	Yes	0.52	1.05	0 36
165614	Yes	0.32	0.82	0 2
167987	Yes	0.58	1.03	0 32
165786		0 56	0.94	0.35
164504				
DNA Synthesis and Repair				
163306		0 57	0.98	0.35
167135	Yes	0 34	0.63	0.2
160035		0.21	0 72	0.11
160262		0 19	0 58	0 12
161026		0 54	0 78	0 28
159889		0.33	0 79	0 22
167553	Yes	0 34	0 67	0 23
163824		0 39	0 79	0 24
169625		0 98	0 88	0 44
Cell Cycle				
167964		0 15	0.33	0 08
160715	Yes	0 33	0.94	0.17
167180		1 54	1.37	1 17
165052		0 18	0.6	0 08
162391		0 17	0.6	0.11

Table 3			
Unique ID No. of Gene	Expression of Genes in Large Cell (LC), Small Cell (SC) and Typical Carcinoma (TC) Cells		
Gene Family (LOH)	LC	SC	TC
162631	0.43	1 06	0 38
168638	0 21	0 58	0 14
Anti-Oncogenes			
161058 Yes	0 72	1 25	0 39
165648	0 31	0 6	0 19
169551	0 47	0 8	0 26
160802	0.16	0 44	0 09
161574 Yes	0 6	1.05	0 4
Oncogenes			
160429	2.54	0 71	0.94
167948 Yes	0.61	1 16	0 28
159898 Yes	0 28	0.42	0 09
165799 Yes	0 53	0.67	0 27
Cytoskeleton/Migration			
160999 Yes	0 42	0 91	0 24
161754	0.53	1.11	0 35
169071 Yes	0 3	0.72	0 21
167351	0 39	0.69	0 26
162343	0 33	0 67	0 17
162727 Yes	0.2	0 45	0 11
165784 Yes	1.46	0.69	1 96
160605	5 94	0.84	1.06
Proteasome			
166328	1 14	0 72	2 12
169691 Yes	0 15	0 34	0.09
168266 Yes	0.2	0 45	0 1
164244 Yes	0.43	0.67	0 22
Drug Resistance			
161117	2 52	0.75	1.12
167738	0.32	0.64	0.18
167505	0 39	0.77	0.21
166359 Yes	0 46	0 64	0 28
167153	6 27	1	1 31
168061	1 32	0 64	1 23
Growth Factors/Receptors And Signal Transduction Enzymes			
165576	1.93	0.66	1.34
169180	1.88	0.86	1.61
160617	3 57	0.86	0 93
164652	2.63	0 97	1 18
165617	2.9	0.73	1 32
169432	2.04	0.65	1.04
161391	0 43	0.83	0.25
164850	0.2	0.45	0.09
165483	0.33	0.98	0.23
162006	0 29	0.71	0.2
161454	0 58	0.99	0.39
168453	0 35	0 59	0 18
162220	0 34	0.76	0.25
160233	2 07	0 97	1 13
Neuronal Markers			
166807			
159877	1 39	0.93	5 89
162207 Yes	0.17	0.58	0.13

Table 3				
Unique ID No. of Gene		Expression of Genes in Large Cell (LC), Small Cell (SC) and Typical Carcinoma (TC) Cells		
Gene Family	(LOH)	LC	SC	TC
161948		0 19	0.56	0 09
159898	Yes	0 28	0 42	0 09
160127	Yes	0 14	0 44	0.1
161955		0.17	0 38	0.09
167591		0.18	0 46	0 14
162006		0 29	0.71	0 2
160887		0 89	1.4	0 56
162247				
165400		1.7	0 76	0.82
RNA Synthesis, Processing and Transcription Factors				
161598		0 82	0.96	2.59
169429		4 52	0 8	1 18
165076		0 96	0 81	1.53
167103		1.7	0.72	1.34
169391	Yes	0 98	0 66	1 15
166789	Yes	1 76	0.75	1 07
168428	Yes			
165588		1 11	0 8	0 57
164927		0 51	1 65	1.4
160630	Yes	0.53	1 15	1 35
160367		0.58	1.26	0.92
167931		0 38	0 99	0 35
161533		1 59	0.67	0 48
168110	Yes	0.35	0.8	0.21
161374	Yes	0.34	0 89	0.19
162587		0.28	0 63	0 17
160467	Yes	0.17	0 44	0.12
160803	Yes	0 3	0 71	0 18
169254	Yes	0 29	0.6	0 16
160678		0 48	0 94	0.29
160242		0 59	0.83	0.31
164106	Yes	0.48	0 61	0 24
166574	Yes	0 47	0 89	0.25
169564		0.25	0 48	0.15
164642		0.69	0.92	0.27
162419		0.59	1.03	0.44
163425		0.95	0.86	0 44
160303	Yes	0 62	1.45	0 46
164573	Yes	2 23	0 82	1.37

Molecular Signature Of The Subtypes Of Pulmonary Neuroendocrine Tumors. The expression profile of genes significantly altered in neuroendocrine tumors was examined to determine whether such information could be used to differentiate among each subtype of pulmonary neuroendocrine tumors. To establish a signature list for each tumor type, the relative expression ratio between TC, LCNEC and SCLC is employed. **Table 4** shows the extent of expression of

such a signature list, and provides the ratio of expression. In **Table 4**, TC/SC denotes genes exhibiting higher levels of expression in TC cells than in SC cells; SC/TC denotes genes exhibiting higher levels of expression in SC cells than in TC cells. Data for TC/LC, LC/TC, SC/LC, and LC/SC are similarly provided. This form of statistical analysis is independent of the reference value and, therefore, can be used for future studies. Using a ratio of 1.9 or higher, it is found that TC had 15 genes whose expression distinguished these tumors from SCLC, and 12 from LCNEC. In contrast, 134 genes are higher in SCLC than in TC and 97 higher than in LCNEC (**Table 4**). The difference between expression of genes in LCNEC from SCLC is encompassed within 34 genes. Thus, cDNA microarray analysis derived expression profile obtained using a cell line as a reference can be used to develop a molecular signature algorithm which may be useful for differential diagnosis of these tumors.

Table 4
Molecular Signature of Neuroendocrine Tumors

Unique ID No. of Gene	Observed Expression		Ratio	Observed Expression
	TC / SC			
	TC	SC	TC/SC	Normal Cells
159877	5.89	0.93	6.33	
167158	6.52	1.16	5.62	
166807	4.46	0 81	5 51	
163031	3 15	1 02	3 09	1 06
166328	2 12	0 72	2.94	
165784	1 96	0.69	2 84	
161598	2.59	0 96	2.70	
165393	1.98	0 96	2 10	
168700	1.69	0 82	2 06	
165192	1 56	0 76	2 05	
165576	1 34	0 66	2 03	
168061	1.23	0 64	1 92	
168276	1 21	0 63	1 92	
165076	1 53	0 81	1 89	
169180	1 61	0 86	1 87	
	SC / TC			
	SC	TC	SC/TC	Normal Cells
165052	0.60	0 08	7.50	0.50
161163	0 53	0.08	6 63	0 40
160035	0 72	0 11	6 55	0 50
161223	0 71	0 11	6 45	0 40
161948	0 56	0.09	6.22	0.22
166635	0.63	0 11	5 73	0 40

Table 4 Molecular Signature of Neuroendocrine Tumors				
Unique ID No. of Gene	Observed Expression		Ratio	Observed Expression
165583	0 28	0 05	5 60	0.20
160715	0.94	0 17	5 53	0 67
162391	0 60	0.11	5 45	0 35
161244	0 38	0 07	5.43	0 20
161211	0 64	0 12	5.33	0.35
161774	0 57	0 11	5.18	0 40
166195	0 56	0 11	5.09	0 30
164850	0 45	0 09	5.00	0.38
160802	0 44	0 09	4 89	
161643	1.16	0 24	4 83	0 80
160262	0.58	0.12	4.83	
164206	0 53	0.11	4 82	0.40
164586	0 48	0 10	4 80	0.35
165039	0 19	0 04	4.75	0 10
161374	0.89	0.19	4 68	0.55
159898	0 42	0 09	4.67	0.26
160102	1 07	0 23	4 65	
164646	0.69	0 15	4 60	0 42
163787	0.81	0.18	4 50	0 50
168266	0.45	0 10	4.50	
161790	0 45	0 10	4 50	
162207	0 58	0 13	4 46	0 55
160127	0 44	0.10	4 40	0 40
160323	0 43	0 10	4 30	0.30
165483	0 98	0 23	4 26	0 73
161955	0.38	0.09	4 22	
167948	1 16	0 28	4.14	1 86
168638	0 58	0 14	4.14	
167964	0 33	0.08	4.13	0.23
166960	0.37	0 09	4.11	0 25
161954	0 78	0.19	4 11	0 20
165614	0.82	0 20	4.10	0 50
162727	0.45	0.11	4 09	0 25
167116	0.32	0.08	4.00	
160803	0.71	0 18	3 94	0.50
162343	0.67	0 17	3 94	0 62
163682	0.59	0.15	3.93	
162623	0.51	0 13	3 92	0.35
166914	0 61	0 16	3 81	
168110	0.80	0 21	3 81	
160999	0 91	0 24	3 79	0.60
160486	0 72	0.19	3 79	0 50
160275	0 53	0.14	3 79	
169691	0 34	0 09	3.78	
165790	0 45	0 12	3.75	0.30
169254	0 60	0 16	3 75	
168079	0 93	0.25	3 72	0 56
162587	0 63	0.17	3.71	0 55
162244	0.74	0 20	3.70	0 70
167505	0 77	0 21	3.67	
160467	0 44	0 12	3 67	0 30
161012	0 73	0 20	3 65	0 55
159889	0 79	0 22	3 59	0.55
163690	0 82	0 23	3 57	0 50
166574	0.89	0 25	3 56	0.62
167738	0 64	0 18	3.56	0 51
167706	0 64	0 18	3 56	
162006	0.71	0.20	3 55	0 31
166010	0.99	0.28	3 54	0 55

Table 4
Molecular Signature of Neuroendocrine Tumors

Unique ID No. of Gene	Observed Expression		Ratio	Observed Expression
167607	0.81	0.23	3.52	0 82
159906	0.62	0 18	3.44	0.30
162150	1 10	0 32	3 44	0 60
169071	0 72	0 21	3 43	
162178	0 24	0.07	3 43	0 20
164642	0.92	0 27	3 41	0.40
167170	0 88	0 26	3 38	0 52
168386	0 81	0.24	3.38	
167223	0.87	0 26	3.35	0.65
161391	0 83	0 25	3 32	0 70
167906	0 63	0.19	3 32	
160565	0.56	0.17	3 29	0 56
163824	0 79	0 24	3 29	
167591	0 46	0 14	3 29	
168453	0 59	0.18	3.28	
161794	0.95	0.29	3.28	0.74
163726	1 21	0.37	3.27	0.90
160038	1.04	0.32	3.25	0.63
160678	0.94	0 29	3.24	
167987	1 03	0 32	3 22	
164504	0 77	0.24	3 21	0 80
161058	1 25	0.39	3.21	
168642	0.96	0.30	3 20	
169564	0 48	0.15	3 20	
165171	0.16	0.05	3.20	
161754	1.11	0 35	3.17	0 60
165648	0.60	0.19	3 16	0 48
162734	1 01	0 32	3 16	0 65
160303	1 45	0.46	3 15	1 30
167135	0 63	0 20	3.15	
160098	0.91	0 29	3 14	0 50
169551	0 80	0 26	3 08	
164244	0 67	0 22	3 05	
162220	0 76	0.25	3.04	0.60
164286	0.94	0 31	3 03	
161635	1 06	0 35	3 03	0 80
167713	0 77	0 26	2 96	
163276	0 47	0.16	2.94	
161178	1 05	0.36	2 92	0 60
167553	0 67	0.23	2 91	
163921	0.52	0 18	2 89	0 55
167931	0 99	0 35	2.83	
160938	0 82	0.29	2.83	0.50
163306	0 98	0.35	2.80	0 50
161650	1.23	0.44	2.80	
162631	1 06	0 38	2 79	
161026	0 78	0.28	2.79	
162571	1.11	0.40	2 78	0 80
160478	1 07	0 39	2 74	
160441	0 49	0.18	2.72	0 42
165786	0 95	0 35	2 71	0 60
165571	0 84	0 31	2 71	0 80
161620	0 84	0 31	2 71	0 80
165813	0 75	0.28	2.68	0 70
160242	0 83	0.31	2.68	
168302	0 88	0.33	2 67	
167351	0.69	0 26	2 65	0 40
168987	0.79	0 30	2.63	
161574	1 05	0 40	2 63	
162510	0 91	0 35	2 60	0.72

Table 4
Molecular Signature of Neuroendocrine Tumors

Unique ID No. of Gene	Observed Expression		Ratio	Observed Expression
164106	0 61	0.24	2 54	0 50
161454	0.99	0.39	2.54	0 60
160887	1 40	0 56	2.50	1.24
165799	0 67	0 27	2 48	0 55
162419	1.03	0.44	2.34	0 80
166359	0 64	0 28	2 29	
169625	0 88	0 44	2 00	
168426	1.09	0.55	1.98	
163425	0.86	0 44	1.95	0 80
	TC / LC			
	TC	**LC**	**TC/LC**	**Normal Cells**
167158	6 52	0 87	7 49	
159877	5.89	1 39	4 24	
166807	4 46	1 11	4 02	
161598	2 59	0 82	3 16	
164927	1 40	0 51	2 75	
163031	3.15	1.22	2.58	
160630	1 35	0 53	2.55	
162247	1 40	0 67	2 09	
167219	1.16	0.57	2.04	
163475	1.17	0 60	1.95	
163762	1 04	0 54	1.93	
166328	2 12	1 14	1 86	
	LC / TC			
	LC	**TC**	**LC/TC**	**Normal Cells**
165400	1 70	0.82	2 07	
164850	0 20	0.09	2.22	
164868	2.39	1.16	2.06	
161533	1 59	0 48	3 31	
160957	3 20	1 16	2 76	
169429	4 52	1.18	3.83	
169432	2 04	1 04	1.96	
165583	0 10	0 05	2 00	0 20
165617	2 90	1 32	2 20	
168987	0.60	0 30	2.00	
161709	1 89	0.79	2 39	
169625	0 98	0 44	2 23	
165799	0 53	0 27	1 96	0 55
161896	2.12	1 04	2.04	
165813	0.59	0 28	2 11	0 70
162571	1 32	0 40	3 30	0.80
161948	0 19	0 09	2 11	0 22
167116	0 18	0.08	2 25	
167125	3 23	1.36	2.38	
167153	6 27	1 31	4 79	
162734	0 73	0 32	2.28	0 60
163425	0 95	0 44	2.16	0.80
164106	0 48	0 24	2 00	0 50
160237	3 50	1.38	2.54	
164206	0 26	0 11	2 36	
164244	0 43	0 22	1 95	
168266	0 20	0 10	2 00	
160429	2 54	0 94	2.70	0.94
159898	0.28	0 09	3.11	0 25
160441	0 37	0 18	2.06	0.42
167713	0 64	0.26	2 46	
165052	0.18	0 08	2.25	0.50
159906	0 42	0 18	2 33	0 30

Table 4
Molecular Signature of Neuroendocrine Tumors

Unique ID No. of Gene	Observed Expression		Ratio	Observed Expression
161117	2.52	1 12	2 25	1 12
161163	0.18	0.08	2 25	0 35
160565	0 45	0 17	2.65	0.50
164504	0 51	0 24	2 13	0 80
165171	0.30	0 05	6.00	
161211	0 27	0.12	2.25	0 35
160605	5.94	1 06	5.60	0.78
160617	3 57	0 93	3 84	0 90
167906	0 40	0.19	2 11	0 80
167948	0 61	0.28	2 18	
164642	0.69	0 27	2.56	0.45
164646	0 39	0 15	2 60	0 42
164652	2 63	1 18	2 23	
	SC / LC			
	SC	**LC**	**SC/LC**	**Normal Cells**
161244	0 38	0 10	3.80	0.20
161223	0 71	0 20	3 55	0.40
162391	0 60	0 17	3 53	0 35
166635	0.63	0.18	3.50	0.40
160035	0 72	0 21	3 43	0 50
162207	0 58	0 17	3 41	0 55
165052	0 60	0 18	3 33	0 50
161954	0.78	0.24	3.25	0 20
164927	1 65	0 51	3 24	
160127	0 44	0 14	3 14	0.47
160262	0.58	0.19	3.05	
161643	1.16	0 39	2.97	0.60
165483	0 98	0 33	2 97	0 73
166195	0 56	0 19	2.95	0.30
161948	0 56	0 19	2 95	0 22
161163	0 53	0.18	2 94	0 35
167223	0 87	0 30	2 90	0.65
161774	0.57	0.20	2.85	0 45
160715	0 94	0 33	2.85	0 67
164586	0 48	0 17	2 82	0 35
161790	0 45	0.16	2 81	
165583	0.28	0.10	2.80	0 20
168638	0 58	0 21	2 76	0.58
160802	0 44	0 16	2 75	
160102	1 07	0 39	2 74	
165039	0 19	0 07	2 71	0 10
163762	1 44	0 54	2 67	
161374	0 89	0 34	2.62	0.55
163787	0 81	0 31	2 61	0 50
161012	0 73	0 28	2 61	0 55
167931	0 99	0 38	2 61	
160467	0 44	0.17	2.59	0.30
165614	0.82	0 32	2.56	0.50
167591	0 46	0 18	2.56	
165790	0 45	0 18	2 50	0 30
162244	0 74	0 30	2.47	0 70
162631	1 06	0 43	2 47	
161635	1 06	0 43	2 47	0 80
162006	0 71	0 29	2 45	0 31
162247	1 62	0.67	2.42	
169071	0 72	0.30	2.40	
159889	0.79	0 33	2.39	0 55
160323	0 43	0.18	2 39	0.30
161211	0 64	0.27	2 37	0.35

Table 4
Molecular Signature of Neuroendocrine Tumors

Unique ID No. of Gene	Observed Expression		Ratio	Observed Expression
160803	0 71	0 30	2 37	0.55
160303	1 45	0 62	2 34	1 00
161794	0 95	0 41	2.32	0.70
168110	0 80	0 35	2 29	
167706	0 64	0 28	2 29	
169691	0 34	0 15	2 27	
168386	0 81	0 36	2.25	
162587	0 63	0 28	2 25	
168266	0.45	0 20	2 25	
164850	0 45	0.20	2.25	0 38
162727	0 45	0.20	2 25	0 25
162220	0.76	0 34	2.24	0.60
161955	0.38	0 17	2 24	
162623	0 51	0.23	2 22	0 36
160038	1 04	0.47	2 21	
167964	0 33	0 15	2 20	
166010	0 99	0 45	2 20	0.55
167170	0.88	0.40	2 20	0.52
167219	1.25	0 57	2.19	
163682	0 59	0.27	2 19	
162178	0 24	0.11	2.18	0.20
166960	0 37	0 17	2 18	0.25
160367	1 26	0 58	2 17	
160630	1.15	0 53	2 17	
160999	0 91	0.42	2 17	0.60
160275	0 53	0 25	2 12	
161754	1.11	0 53	2.09	0 60
163921	0 52	0.25	2 08	0.55
169254	0 60	0 29	2 07	0.28
164206	0 53	0 26	2 04	0 40
166914	0 61	0 30	2 03	
162343	0.67	0.33	2.03	0 62
163824	0 79	0.39	2 03	0.65
167607	0 81	0.40	2 03	
160098	0.91	0 45	2.02	0 50
168079	0.93	0 46	2.02	0 56
161178	1 05	0.52	2 02	0.60
160938	0.82	0.41	2 00	0.50
167738	0 64	0 32	2 00	0.51
167505	0 77	0 39	1 97	
159859	1 44	0.73	1 97	0 90
167553	0.67	0 34	1.97	
162150	1 10	0.56	1 96	
160678	0 94	0.48	1 96	
163690	0.82	0 42	1.95	0.50
160486	0 72	0.37	1 95	0 50
160478	1 07	0.55	1 95	
165648	0 60	0 31	1 94	
161391	0.83	0 43	1 93	0 70
169564	0 48	0.25	1 92	
167948	1 16	0.61	1 90	
166574	0 89	0 47	1 89	
167135	0.63	0 34	1 85	
	LC / SC			
	LC	**SC**	**LC/SC**	**Normal Cells**
165393	2 66	0.96	2.77	
168700	1 91	0.82	2 33	
169384	2 28	0 77	2 96	
165400	1 70	0 76	2 24	

Table 4
Molecular Signature of Neuroendocrine Tumors

Unique ID No. of Gene	Observed Expression		Ratio	Observed Expression
161533	1.59	0 67	2 37	1.00
160957	3 20	0 77	4.16	
169429	4 52	0 80	5.65	
169432	2.04	0 65	3.14	
165576	1 93	0.66	2.92	
165617	2 90	0.73	3 97	
161709	1 89	0 95	1 99	
165784	1 46	0 69	2.12	
162475	2 00	1 06	1.89	
161896	2 12	0.75	2 83	
167103	1 70	0 72	2 36	
167125	3 23	0 88	3 67	
167153	6.27	1 00	6 27	
167316	1 94	0.88	2 20	
166789	1 76	0 75	2 35	
168061	1.32	0 64	2 06	
160233	2.07	0 97	2.13	
160237	3 50	0 92	3 80	
168141	2.51	0 95	2 64	
168169	2.78	1 17	2.38	
168276	1 61	0.63	2.56	
159813	1 99	0 83	2 40	
160429	2 54	0.71	3 58	0.90
161117	2 52	0 75	3 36	
165171	0 30	0 16	1.88	
164573	2.23	0.82	2.72	
160605	5 94	0 84	7.07	0 78
160617	3 57	0.86	4 15	0 90
169180	1.88	0.86	2 19	
164652	2 63	0.97	2 71	

Correlation Between Gene Expression Profiles And Genomic Imbalance. To compare the results obtained from cDNA array expression in accordance with the present invention with previously available information on genomic imbalances in neuroendocrine tumors, a search of the literature for published data on comparative genomic hybridization (CGH) and loss of heterozygosity (LOH) in neuroendocrine tumors was conducted. It was found that, among 198 genes identified by the Class Comparison (F-test) analysis, over ninety percent of genes with significant changes in LCNEC, and over 80% of genes from SCLC and TC, had previously been reported to have chromosomal imbalances by gain or loss (CGH) or to be associated with LOH (**Table 5**). Loss of chromosomal material by LOH closely correlated with genes whose expression significantly decreased in our analysis. Deletions of several genes, such as cyclin-dependent kinase inhibitor (CDKN2A, 9p21) and multiple endocrine neoplasia 1 (MEN1,

11q13) have been studied extensively in pulmonary neuroendocrine tumors (Oliveira, A.M. *et al.* (2001) "FAMILIAL PULMONARY CARCINOID TUMORS," Cancer 91:2104-2109; Debelenko, L.V. *et al.* (2000) "MEN1 gene mutation analysis of high-grade neuroendocrine lung carcinoma," Genes Chromosomes Cancer. 28:58-65). However, several genes whose expression has been found to be decreased herein were previously reported to have a gain of chromosomal material by CGH. These include BAK, excision repair cross-complement (ERCC1), DNA ligase (LIG1), tubulin beta (TUBB) and others (**Table 2**).

Of interest, none of the genes which encode for growth factor/receptors identified herein have been reported by LOH. However, loss of genetic material by CGH in these genes has been reported. The potential loss of repressor activity in the promoter regions of these genes may result in their over-expression as detected herein. In sum, the expression profiling of significantly altered genes derived from microarray data reported herein closely correlates with chromosomal imbalances reported by LOH but not by CGH.

Example 3
Analysis of Gene Expression Profiles

Analysis of clusters of differentially expressed mRNAs from 9,984 human transcripts assigned to each subtype of neuroendocrine tumors identified multiple genes (198 genes with a probability of 0.004) exhibiting differential expression. This highly selected group of genes contained valuable information which correlated with biological behavior of these tumors. The identified genes are involved in regulation of apoptosis, cell-cell and cell-matrix interactions, cell cycle, DNA synthesis and repair, drug resistance, RNA synthesis and processing, receptors and growth factors. Previous studies using microarray analysis of lymphomas (Dodson, J.M. *et al.* (2002) "QUANTITATIVE ASSESSMENT OF FILTER-BASED CDNA MICROARRAYS: GENE EXPRESSION PROFILES OF HUMAN T-LYMPHOMA CELL LINES," Bioinformatics 18:953-960; Ramaswamy, S. *et al.* (2001) MULTICLASS CANCER DIAGNOSIS USING TUMOR GENE EXPRESSION SIGNATURES," Proc Natl Acad Sci U S A. 98(26):15149-15154), gastrointestinal (Hippo, Y. etal. (2002) "GLOBAL GENE EXPRESSION ANALYSIS OF GASTRIC

CANCER BY OLIGONUCLEOTIDE MICROARRAYS," Cancer Res. 62(1):233-240; Selaru, F.M. *et al.* (2002) "ARTIFICIAL NEURAL NETWORKS DISTINGUISH AMONG SUBTYPES OF NEOPLASTIC COLORECTAL LESIONS," Gastroenterology 122:606-613), ovarian (Ramaswamy, S. *et al.* (2001) MULTICLASS CANCER DIAGNOSIS USING TUMOR GENE EXPRESSION SIGNATURES," Proc Natl Acad Sci U S A. 98(26):15149-15154), and other types of human tumors found that over-expression of specific genes is a prominent feature that facilitated the molecular classification of these tumors. In contrast, a significant decrease in expression in the majority of the selected genes was found. One of the major survival pathways is regulated by protection of the mitochondrial membrane by BCL2 which is frequently over-expressed in tumor cells (Cleary, M.L. *et al.* (1986) "CLONING AND STRUCTURAL ANALYSIS OF CDNAS FOR BCL-2 AND A HYBRID BCL-2/IMMUNOGLOBULIN TRANSCRIPT RESULTING FROM THE T(14;18) TRANSLOCATION," Cell. 47(1):19-28). Decreased expression of BCL2 antagonists, BAD and BAK1 was observed in samples from TC and LCNEC. This feature may provide survival advantage without the need for over-expression of BCL2 as occurs in certain types of lymphomas. BAD and BAK1 are located on chromosomes 11q13 and 6p21, respectively, which are in the regions of loss of heterozygosity (LOH) in neuroendocrine tumors (Hofmann, W.K. (2002) "RELATION BETWEEN RESISTANCE OF PHILADELPHIA-CHROMOSOME-POSITIVE ACUTE LYMPHOBLASTIC LEUKAEMIA TO THE TYROSINE KINASE INHIBITOR STI571 AND GENE-EXPRESSION PROFILES: A GENE-EXPRESSION STUDY," Lancet 359:481-486). Expression of BAK was further suppressed in TC and LCNEC below the level expected for LOH which suggests an additional regulatory mechanism. Interestingly, gain of chromosomal material in 6p21 was reported in LCNEC by CGH (Michelland, S. *et al.* (1999) "COMPARISON OF CHROMOSOMAL IMBALANCES IN NEUROENDOCRINE AND NON-SMALL-CELL LUNG CARCINOMAS," Cancer Genet Cytogenet 114:22-30). Suppression of other apoptosis-promoting genes, such as caspase 4 (CASP4), may also provide survival advantage and has not been previously reported in Neuroendocrine tumors. Loss of expression of many genes which regulate cell-cell and cell-matrix interactions as well as DNA and RNA synthesis and repair were

apparent in all tumor types (**Table 2**). **Table 2** shows representative deregulated genes classified by function. Genes selected by F-test with probability of <0.004 were genes assigned to functional categories and compared with the published comparative genomic hybridization (CGH) results (Michelland, S. *et al.* (1999) "COMPARISON OF CHROMOSOMAL IMBALANCES IN NEUROENDOCRINE AND NON-SMALL-CELL LUNG CARCINOMAS," Cancer Genet Cytogenet 114:22-30; Lui, W.-O. *et al.* (2001) "HIGH LEVEL AMPLIFICATION OF 1P32-33 AND 2P22-24 IN SMALL CELL LUNG CARCINOMAS" *Intl. J Oncol. 19*:451-457; Ullmann, R., *et al.* (2001) "CHROMOSOMAL ABERRATIONS IN A SERIES OF LARGE-CELL NEUROENDOCRINE CARCINOMAS: UNEXPECTED DIVERGENCE FROM SMALL-CELL CARCINOMA OF THE LUNG," Hum Pathol. 32:1059-63; Walch, A.K. *et al.* (1998) "TYPICAL AND ATYPICAL CARCINOID TUMORS OF THE LUNG ARE CHARACTERIZED BY 11Q DELETIONS AS DETECTED BY COMPARATIVE GENOMIC HYBRIDIZATION" *Am J Pathol. 153*:1089-98).

In the table, SC denotes small cell; LC denotes large cell neuroendocrine carcinoma; and TC denotes typical carcinoid.

Most studies performed to-date compare tumor samples with cDNA from normal tissues of an individual patient, pooled normal tissues or pooled cell lines as reference. To illustrate the invention, RNA from a single human cell line derived from normal bronchial epithelium, BEAS-2B (Amstad, P. *et al.* (1988) "NEOPLASTIC TRANSFORMATION OF A HUMAN BRONCHIAL EPITHELIAL CELL LINE BY A RECOMBINANT RETROVIRUS ENCODING VIRAL HARVEY RAS," Mol Carcinog. 1988 1:151-60), was used as a reference RNA. This cell line has minimal chromosomal rearrangements in early passages and neuroendocrine tumor features (Lee, B.H *et al.* (1998) "IN VITRO CHROMOSOME ABERRATION ASSAY USING HUMAN BRONCHIAL EPITHELIAL CELLS," J. Toxicol Environ. Health A. 55:325-9). Thus, the data indicate that accurate classification of neuroendocrine tumors can be achieved by comparing gene expression profiles of tumors to a single cell line derived from the same cell type. This method is applicable to analysis of tumor-derived gene expression profiles from other organs, such as brain, where availability of normal tissue is limited.

In addition to suppression of the apoptotic pathway, only LCNEC tumors had increased expression (2-6- fold) of several receptors and growth factors. Increased expression of PDGFRB in conjunction with suppression of PDGFA-associated protein, which can down regulate the activity of PDGFA, could result in additional proliferative signal and contribute to the aggressive behavior of this tumor. In addition, high expression of an adhesion plaque-associated protein, P311, which has been recently identified as a glioblastoma invasion gene (Mariani, L. *et al.* (2001) "IDENTIFICATION AND VALIDATION OF P311 AS A GLIOBLASTOMA INVASION GENE USING LASER CAPTURE MICRODISSECTION," Cancer Res 61:4190-4196) was detected.

The lack of a similar pattern of gene expression in SCLC may result from the small number of samples examined or may result from different transforming mechanisms since oncogenic mutations ($p21^{ras}$, p53 and others) but not over-expressions are associated with SCLC (Wistuba, I.I. *et al.* (2001) "MOLECULAR GENETICS OF SMALL CELL LUNG CARCINOMA," Semin Oncol 28: 3-13). Functional analysis of genes whose expression significantly altered in pulmonary neuroendocrine tumors provides insight into the underlying biological mechanism, leading to survival and slow progression of TC whereas LCNEC and SCLC have an aggressive behavior.

Many studies have identified genes whose expression is significantly suppressed in neuroendocrine tumors. High incidence of LOH at 3p, 5q, 11q, and 17p (Ohnuki, Y. *et al.* (1996) "CHROMOSOMAL CHANGES AND PROGRESSIVE TUMORIGENESIS OF HUMAN BRONCHIAL EPITHELIAL CELL LINES," Cancer Genet. Cytogenet. 92:99-110), except for chromosome 13q, correlates with significant decrease in expression of genes assigned to these locations, including MENI (11q13). The data adds to previously reported studies and confirms that expression profiling of lung neuroendocrine tumors provides accurate tumor classification. The molecular signature of relative abundance of gene expression derived by comparing mean gene expression of each 3 tumor subtypes is independent of the reference RNA and is of particular interest because of its clinical relevance. These results indicate that gene expression profiling of pulmonary neuroendocrine tumors

provides a diagnostic tool for tumor classification, particularly when histopathology interpretation is ambiguous.

In summary, light microscopy-based classification of pulmonary neuroendocrine tumors is often difficult. To search for molecular markers of neuroendocrine tumors, cDNA microarrays of 9,984 human transcripts were used to identify classification-associated genes at a global genomic scale. Laser-capture microdissection was used to harvest tumor cells from frozen sections. The gene expression profiles in primary pulmonary neuroendocrine tumors from 17 surgical specimens (11 Typical Carcinoids,(TC), 3 Small Cell lung cancers (SCLC), 2 Large Cell Neuroendocrine tumors (LNEC), and one sample which had features of SCLC and LNEC) were compared. The BRB ArrayTool (National Cancer Institute, NIH; http://linus.nci.nih.gov/BRB-ArrayTools.html) was employed to analyze gene expression patterns. An unsupervised, hierarchical clustering algorithm used to analyze these 17 tumors based only on similarities in gene expression resulted in a precise classification of each tumor type. The Class Comparison Tool used to compare each tumor type identified 198 statistically significant genes ($p<0.004$) that accurately discriminated between 3 pre-defined tumor types. Analysis of these genes revealed that deletions were more frequent than were amplifications in pulmonary neuroendocrine tumors. Using comparative analysis of gene expression variance, a molecular signature for each tumor type was identified. The signature genes included decreased expression of pro-apoptotic genes, cell-cell and cell matrix interacting components, cell cycle control and DNA repair, and anti-oncogenes. In particular, decreased expression of the BCL2 antagonist, BAK1, was found in all tumor types, whereas BAD was decreased in LCNEC and TC tumors. Over-expression of several growth factors and receptors (CSF2RB, PDGFRB, IL13RA2, and IL6ST (gpl30)) was detected only in LCNEC tumors, and increased expression of IL-8Rβ was shared by TC tumor cells. High expression of a neuronal marker, P311, previously reported to promote invasive phenotype in brain tumors, was detected in LCNEC, and a peptide processing enzyme, Carboxypeptidase E (CPE), was found in TC. The analysis indicates that functional genomic comparison of expression profiles can

accurately classify pulmonary neuroendocrine tumors and will therefore facilitate the development of new therapies for patients having these malignancies.

Table 5 lists genes that are differentially expressed in different neuroendocrine tumors.

Table 5

Genes Differentially Expressed In Small Cell Lung Cancer (SCLC) Neuroendocrine Tumor Cells Relative To Large Cell Neuroendocrine Carcinoma (LCNEC) Neuroendocrine Tumor Cells

IncytePD:523635	IncytePD:1734113	IncytePD:2074154
IncytePD:561992	IncytePD:1743234	IncytePD:2104145
IncytePD:605019	IncytePD:1749727	IncytePD:2172334
IncytePD:614679	IncytePD:1755793	IncytePD:2180031
IncytePD:629077	IncytePD:1808260	IncytePD:2182907
IncytePD:637639	IncytePD:1810821	IncytePD:2200079
IncytePD:696002	IncytePD:1821971	IncytePD:2205246
IncytePD:740878	IncytePD:1824957	IncytePD:2308525
IncytePD:771715	IncytePD:1841920	IncytePD:2356635
IncytePD:820580	IncytePD:1853163	IncytePD:2374294
IncytePD:849425	IncytePD:1857493	IncytePD:2469592
IncytePD:942207	IncytePD:1872067	IncytePD:2506427
IncytePD:958513	IncytePD:1890919	IncytePD:2507648
IncytePD:961082	IncytePD:1921567	IncytePD:2508570
IncytePD:998069	IncytePD:1931265	IncytePD:2568547
IncytePD:1258790	IncytePD:1942845	IncytePD:2610374
IncytePD:1297269	IncytePD:1960722	IncytePD:2663948
IncytePD:1308112	IncytePD:1968721	IncytePD:2674277
IncytePD:1339241	IncytePD:1988239	IncytePD:3038508
IncytePD:1382374	IncytePD:1990361	IncytePD:3115514
IncytePD:1402615	IncytePD:1997937	IncytePD:3123858
IncytePD:1405652	IncytePD:1997967	IncytePD:3179113
IncytePD:1431819	IncytePD:2048144	IncytePD:3202075
IncytePD:1435374	IncytePD:2050085	IncytePD:3255437
IncytePD:1445203	IncytePD:2054529	IncytePD:3333130
IncytePD:1453450	IncytePD:2055640	IncytePD:3360476
IncytePD:1481225	IncytePD:2055687	IncytePD:3381870
IncytePD:1486983	IncytePD:2055773	IncytePD:3427560
IncytePD:1501080	IncytePD:2055926	IncytePD:3432534
IncytePD:1555545	IncytePD:2056149	IncytePD:3518380
IncytePD:1561352	IncytePD:2056172	IncytePD:3562795
IncytePD:1567995	IncytePD:2056987	IncytePD:3842669
IncytePD:1603584	IncytePD:2057547	IncytePD:3967780
IncytePD:1610083	IncytePD:2057823	IncytePD:3990209
IncytePD.1624024	IncytePD:2058537	IncytePD:3999291
IncytePD:1625169	IncytePD:2060308	IncytePD:4014715
IncytePD:1635008	IncytePD:2679117	IncytePD:4016254
IncytePD:1637517	IncytePD:2740235	IncytePD:4059193
IncytePD:1653911	IncytePD:2751387	IncytePD:4144001
IncytePD:1685342	IncytePD:2852403	IncytePD:4287342
IncytePD:1691161	IncytePD:2956581	IncytePD:4626895
IncytePD:1699149	IncytePD:2956906	IncytePD:5017148

IncytePD:1702266	IncytePD:3032691	IncytePD:5096975
IncytePD:1969563	IncytePD:3032825	

Genes Differentially Expressed In Small Cell Lung Cancer (SCLC) Neuroendocrine Tumor Cells Relative To Typical Carcinoid (TC) Neuroendocrine Tumor Cells

IncytePD:477045	IncytePD:1748705	IncytePD:2453436
IncytePD:478960	IncytePD:1749727	IncytePD:2469592
IncytePD:523635	IncytePD:1755793	IncytePD:2506427
IncytePD:557451	IncytePD:1773638	IncytePD:2508570
IncytePD:561992	IncytePD:1807294	IncytePD:2610374
IncytePD:588157	IncytePD:1808260	IncytePD:2622566
IncytePD:605019	IncytePD:1810821	IncytePD:2663948
IncytePD:696002	IncytePD:1812955	IncytePD:2674277
IncytePD:740878	IncytePD:1822716	IncytePD:2679117
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IncytePD:961082	IncytePD:1890919	IncytePD:2798872
IncytePD:1240748	IncytePD:1920650	IncytePD:2806778
IncytePD:1258790	IncytePD:1921567	IncytePD:2852403
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IncytePD:1308112	IncytePD:1942845	IncytePD:2914719
IncytePD:1402615	IncytePD:1960722	IncytePD:2923082
IncytePD:1405652	IncytePD:1968721	IncytePD:2956906
IncytePD:1431819	IncytePD:1988239	IncytePD:3010959
IncytePD:1435374	IncytePD:1997792	IncytePD:3032691
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IncytePD:1453450	IncytePD:2054529	IncytePD:3038508
IncytePD:1481225	IncytePD:2055640	IncytePD:3115514
IncytePD:1486983	IncytePD:2055687	IncytePD:3123858
IncytePD:1488021	IncytePD:2055773	IncytePD:3179113
IncytePD:1505977	IncytePD:2055926	IncytePD:3202075
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IncytePD:1562658	IncytePD:2056987	IncytePD:3518380
IncytePD:1567995	IncytePD:2057547	IncytePD:3562795
IncytePD:1603584	IncytePD:2057823	IncytePD:3728255
IncytePD:1610083	IncytePD:2057908	IncytePD:3805046
IncytePD:1624024	IncytePD:2058537	IncytePD:3871545
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IncytePD:1693847	IncytePD:2182907	IncytePD:4144001
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IncytePD:1702266	IncytePD:2356635	IncytePD:4626895
IncytePD:1704168	IncytePD:2369544	IncytePD:5017148
IncytePD:1712663	IncytePD:2374294	IncytePD:5096975
IncytePD:1734113	IncytePD:2383065	

Genes Differentially Expressed In Large Cell Neuroendocrine Carcinoma (LCNEC) Neuroendocrine Tumor Cells Relative To Typical Carcinoid (TC) Neuroendocrine Tumor Cells		
IncytePD:629077	IncytePD:1748705	IncytePD:2507648
IncytePD:637639	IncytePD:1773638	IncytePD:2508570
IncytePD:818568	IncytePD:1807294	IncytePD:2622566
IncytePD:885601	IncytePD:1812955	IncytePD:2679117
IncytePD:899102	IncytePD:1821971	IncytePD:2728840
IncytePD:942207	IncytePD:1822716	IncytePD:2806778
IncytePD:1308112	IncytePD:1858365	IncytePD:2888814
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IncytePD:1435374	IncytePD:1990361	IncytePD:2956581
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IncytePD:1501080	IncytePD:2048144	IncytePD:3333130
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IncytePD:1555545	IncytePD:2205246	IncytePD:3427560
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IncytePD:1712663	IncytePD:2469592	
IncytePD:1743234	IncytePD:2506427	

The methods employed in the present invention can be similarly employed to facilitate the diagnosis of other tumor types, for example, adenocarcinomas, which are distinct from neuroendocrine tumors and exhibit significant differences in gene expression (Garber, M. E. *et al.* (2001) "DIVERSITY OF GENE EXPRESSION IN ADENOCARCINOMA OF THE LUNG" *Proc. Natl. Acad. Sci. (U.S.A.) 98*:13784–13789; Bhattacharjee, A. *et al.* (2001) "CLASSIFICATION OF HUMAN LUNG CARCINOMAS BY MRNA EXPRESSION PROFILING REVEALS DISTINCT ADENOCARCINOMA SUBCLASSES" Proc. Natl. Acad. Sci. (U.S.A.) 98:13790–13795). cDNA microarrays that can be used to identify profiles of genes expressed in adenocarcinomas are disclosed by Miura, K. *et al.* (2002) ("LASER CAPTURE MICRODISSECTION AND MICROARRAY EXPRESSION ANALYSIS OF LUNG ADENOCARCINOMA REVEALS TOBACCO SMOKING- AND PROGNOSIS-RELATED MOLECULAR PROFILES," Canc. Res. 62:3244-3250).

All publications and patents mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. Having now generally described the invention, the same will be more

readily understood through reference to the following examples, which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth.

SEQUENCE LISTING

<110> Harris, Curtis C..
He, Ping
Varticovski, Lyuba
Travis, William

<120> Methods and Compositions for the Diagnosis of Neuroendocrine Lung Cancer

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What Is Claimed Is:

1. A method for determining whether a candidate cell is a neuroendocrine tumor cell, wherein said method comprises the steps of:

(A) determining the profile of expression of a plurality of genes of said candidate cell; and

(B) comparing such determined profile of expression with the profile of expression of said genes of a small cell lung cancer cell, a large cell neuroendocrine carcinoma cell, a typical carcinoid tumor cell or an atypical carcinoid tumor cell;

to thereby determine whether said candidate cell is a neuroendocrine tumor cell.

2. The method of claim 1, wherein said method additionally permits a determination of neuroendocrine tumor cell type.

3. The method of claim 2, wherein said method determines whether said candidate cell is a small cell lung cancer (SCLC) neuroendocrine tumor cell.

4. The method of claim 2, wherein said method determines whether said candidate cell is a large cell neuroendocrine carcinoma (LCNEC) neuroendocrine tumor cell.

5. The method of claim 2, wherein said method determines whether said candidate cell is a typical carcinoid (TC) neuroendocrine tumor cell.

6. The method of claim 2, wherein said method determines whether said candidate cell is an atypical carcinoid (AT) neuroendocrine tumor cell.

7. The method of claim 2, wherein said step (A) comprises incubating RNA of said candidate cell, or DNA or RNA amplified from such RNA, in the presence of a plurality of genes, or fragments or RNA transcripts thereof,

under conditions sufficient to cause RNA to hybridize to complementary DNA or RNA molecules; and detecting hybridization that occurs.

8. The method of claim 7, wherein said plurality of genes, or polynucleotide fragments or RNA transcripts thereof, are distinguishably arrayed in a microarray.

9. The method of claim 8, wherein said microarray comprises arrayed genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in neuroendocrine tumor cells relative to normal cells.

10. The method of claim 8, wherein said microarray comprises arrayed genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in small cell lung cancer (SCLC) neuroendocrine tumor cells relative to large cell neuroendocrine carcinoma (LCNEC) neuroendocrine tumor cells.

11. The method of claim 8, wherein said microarray comprises arrayed genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in small cell lung cancer (SCLC) neuroendocrine tumor cells relative to typical carcinoid (TC) neuroendocrine tumor cells.

12. The method of claim 8, wherein said microarray comprises arrayed genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in small cell lung cancer (SCLC) neuroendocrine tumor cells relative to atypical carcinoid (AT) neuroendocrine tumor cells.

13. The method of claim 8, wherein said microarray comprises arrayed genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in large cell neuroendocrine carcinoma (LCNEC) neuroendocrine tumor cells relative to typical carcinoid (TC) neuroendocrine tumor cells.

14. The method of claim 8, wherein said microarray comprises arrayed genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in large cell neuroendocrine carcinoma (LCNEC) neuroendocrine tumor cells relative to atypical carcinoid (AT) neuroendocrine tumor cells.

15. The method of claim 8, wherein said microarray comprises arrayed genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in typical carcinoid (TC) neuroendocrine tumor cells relative to atypical carcinoid (AT) neuroendocrine tumor cells.

16. A microarray of genes, or polynucleotide fragments or RNA transcripts thereof for distinguishing a neuroendocrine tumor cell, said microarray comprising a solid support having greater than 10 genes, or polynucleotide fragments or RNA transcripts thereof, distinguishably arrayed in spaced apart regions, wherein said microarray comprises a sufficient number of genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in a small cell lung cancer (SCLC) cell, a large cell neuroendocrine carcinoma (LCNEC) neuroendocrine tumor cell, a typical carcinoid (TC) neuroendocrine tumor cell, or an atypical carcinoid (AT) neuroendocrine tumor cell, relative to a normal cell or a cell belonging to a different neuroendocrine tumor cell type, to permit said microarray to distinguish a neuroendocrine tumor cell.

17. The microarray of claim 16, wherein said microarray comprises a sufficient number of genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in a neuroendocrine tumor cell relative to a normal cell to permit said microarray to distinguish between a neuroendocrine tumor cell and a normal cell.

18. The microarray of claim 16, wherein said microarray comprises a sufficient number of genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in a small cell lung cancer (SCLC)

neuroendocrine tumor cell relative to a large cell neuroendocrine carcinoma (LCNEC) neuroendocrine tumor cell to permit said microarray to distinguish between a small cell lung cancer (SCLC) neuroendocrine tumor cell and a large cell neuroendocrine carcinoma (LCNEC) neuroendocrine tumor cell.

19. The microarray of claim 16, wherein said microarray comprises a sufficient number of genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in a small cell lung cancer (SCLC) neuroendocrine tumor cell relative to a typical carcinoid (TC) neuroendocrine tumor cell to permit said microarray to distinguish between a small cell lung cancer (SCLC) neuroendocrine tumor cell and a typical carcinoid (TC) neuroendocrine tumor cell.

20. The microarray of claim 16, wherein said microarray comprises a sufficient number of genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in a small cell lung cancer (SCLC) neuroendocrine tumor cell relative to an atypical carcinoid (AT) neuroendocrine tumor cell to permit said microarray to distinguish between a small cell lung cancer (SCLC) neuroendocrine tumor cell and an atypical carcinoid (AT) neuroendocrine tumor cell.

21. The microarray of claim 16, wherein said microarray comprises a sufficient number of genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in a large cell neuroendocrine carcinoma (LCNEC) neuroendocrine tumor cell relative to a typical carcinoid (TC) neuroendocrine tumor cell to permit said microarray to distinguish between a large cell neuroendocrine carcinoma (LCNEC) neuroendocrine tumor cell and a typical carcinoid (TC) neuroendocrine tumor cell.

22. The microarray of claim 16, wherein said microarray comprises a sufficient number of genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in a large cell neuroendocrine carcinoma

(LCNEC) neuroendocrine tumor cell relative to an atypical carcinoid (AT) neuroendocrine tumor cell to permit said microarray to distinguish between a large cell neuroendocrine carcinoma (LCNEC) neuroendocrine tumor cell and an atypical carcinoid (AT) neuroendocrine tumor cell.

23. The microarray of claim 16, wherein said microarray comprises a sufficient number of genes, or polynucleotide fragments or RNA transcripts thereof, that are differentially expressed in a typical carcinoid (TC) neuroendocrine tumor cell relative to an atypical carcinoid (AT) neuroendocrine tumor cell to permit said microarray to distinguish between a typical carcinoid (TC) neuroendocrine tumor cell and an atypical carcinoid (AT) neuroendocrine tumor cell.

Abstract of the Invention:

This invention relates to methods and compositions for the diagnosis of neuroendocrine lung cancers. In particular, the invention concerns the use of cDNA microarrays to facilitate the differential diagnosis of neuroendocrine tumor types..

Figure 1A

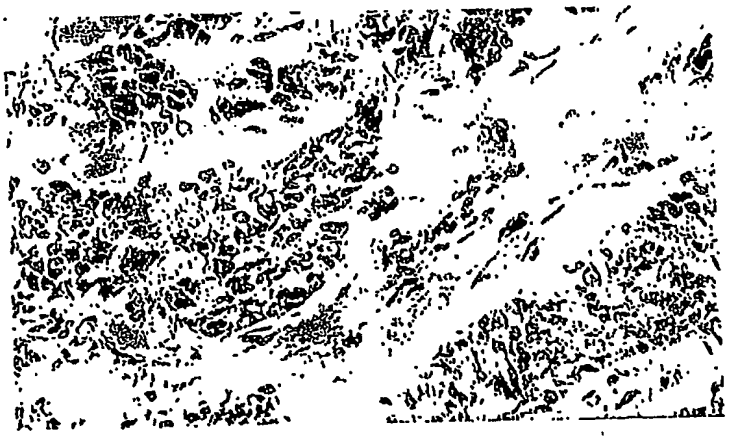


Figure 1B

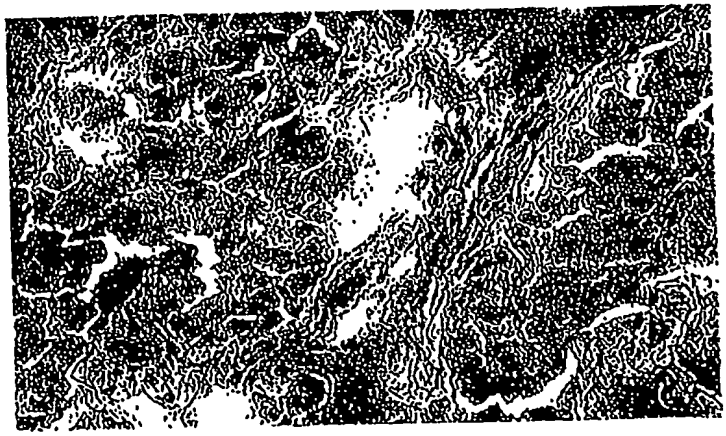
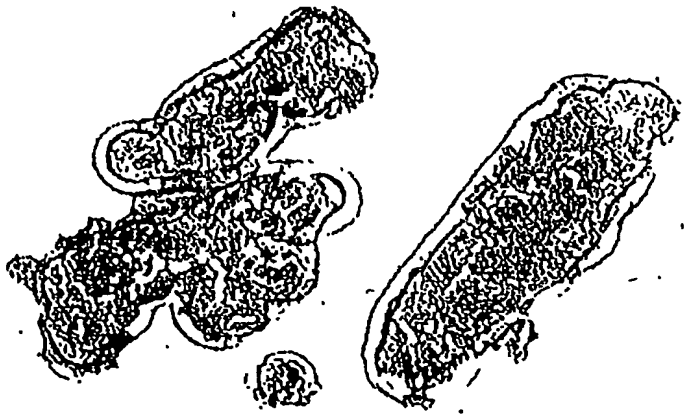


Figure 1C

Figure 1D



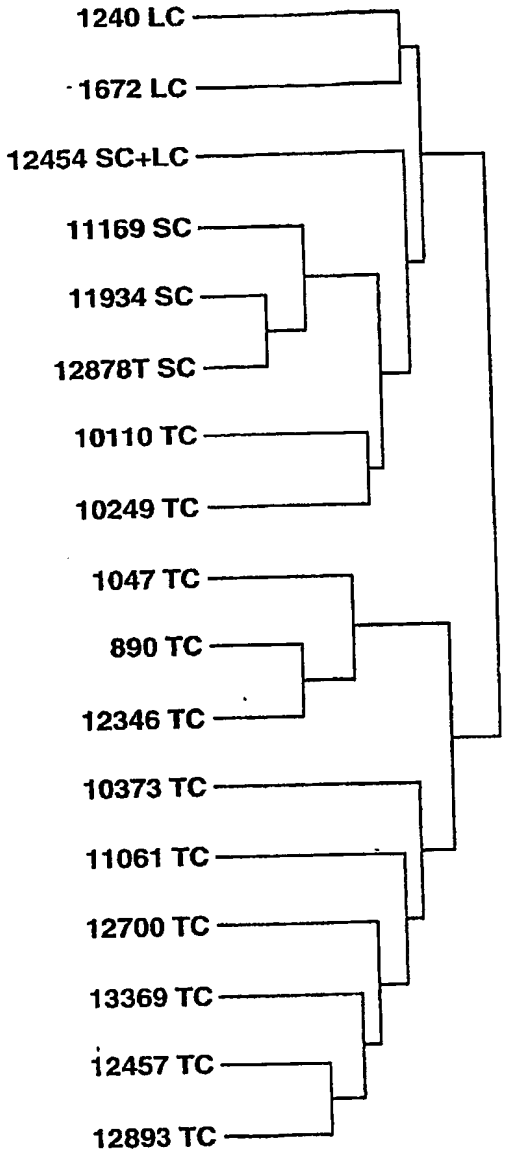

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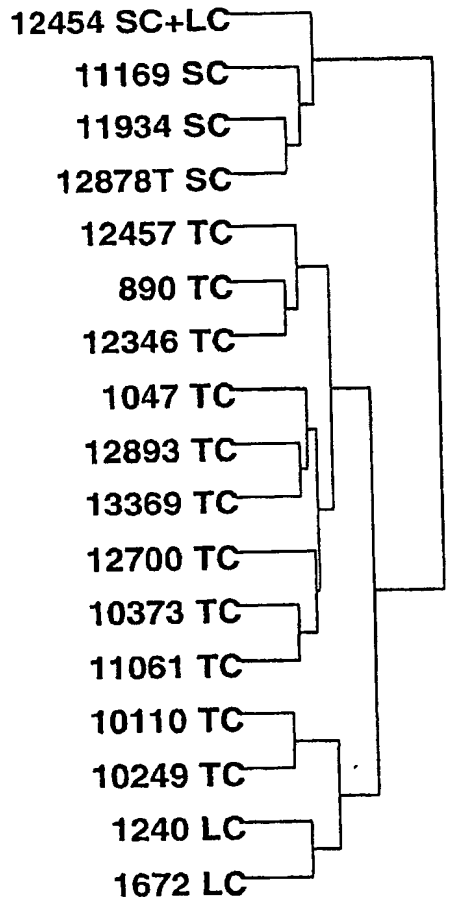
Figure 2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12346 TC
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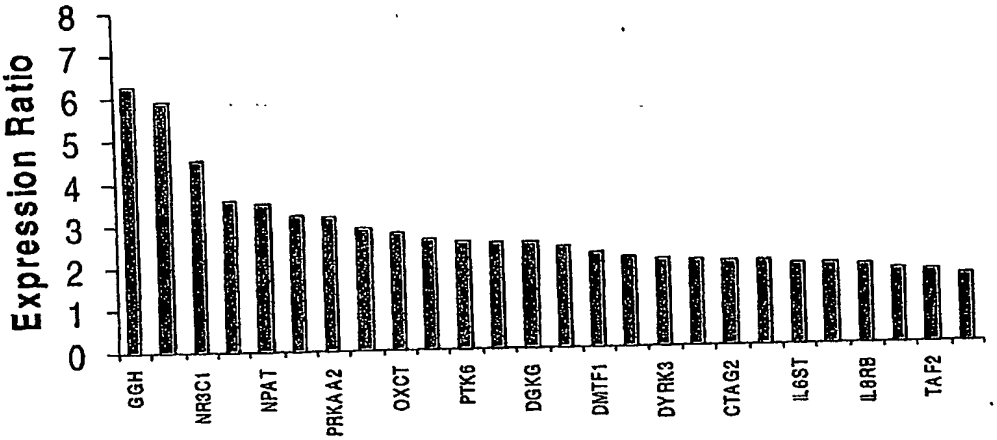
1240 LC
1672 LC

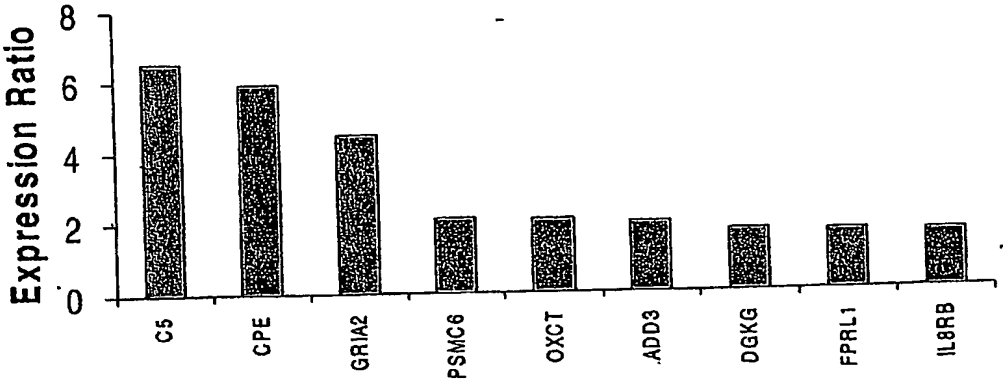
Figure 3

Figure 4A



Genes Overexpressed in LCNEC Tumors

Figure 4B



Genes Overexpressed in TC Neuroendocrine Tumors

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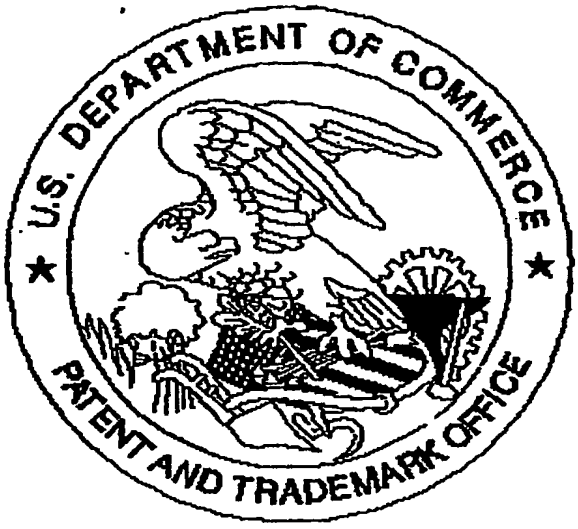

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